Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention

Contract No 07.0202/2018/788519/ETU/ENV.D2

Final Report, executive summary and Annexes

EUROPEAN COMMISSION

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Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention
Contract No 07.0202/2018/788519/ETU/ENV.D2

Period: 18 November 2018 – 17 November 2019

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Abstract: Evidence-based assessments of the risks posed by invasive alien species (IAS) are critical to underpin policies and prioritise action. Over the last three years, a template for producing structured risk assessments has been developed and tested for thirty selected species spanning various taxonomic groups across marine, terrestrial and freshwater environments. Here we present ten risk assessments and associated management annexes for IAS produced over the last year. The selected species were *Channa argus* (northern snakehead), *Ameiurus melas* (black bullhead), *Ameiurus nebulosus* (brown bullhead), *Wasmannia auropunctata* (little fire ant), *Axis axis* (chital), *Pycnonotus cafer* (red-vented bulbul), *Fallopia baldschuanica* (Russian vine), *Phytolacca americana* (American pokeweed), *Boccardia proboscidea* (polychaete worm), *Schizoporella japonica* (orange ripple bryozoan). The risk assessments and associated management annexes were produced alongside tasks to develop and maintain a risk assessment template and to collect evidence on management techniques, implementation costs and cost-effectiveness. A two-day workshop was held to finalise the risk assessments following peer-review. The risk assessments will be used as evidence to inform whether the target species should be considered for inclusion on the list of invasive alien species of Union concern under Regulation (EU) 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (the IAS Regulation).

Résumé: Les évaluations de risques d'espèces exotiques envahissantes (EEE) basées sur des éléments probants sont essentielles pour étayer les politiques et établir les priorités d'action. Au cours des trois dernières années, un modèle pour la production d'évaluations structurées des risques a été élaboré et mis à l'essai pour trente espèces sélectionnées, couvrant divers groupes taxonomiques dans des environnements marins, terrestres et d'eau douce. Nous présentons ici dix évaluations des risques et les annexes de gestion connexes pour les EEE produites au cours de la dernière année. Les espèces sélectionnées étaient *Channa argus* (poisson à tête de serpent), *Ameiurus melas* (poisson-chat), *Ameiurus nebulosus* (barbotte brune), *Wasmannia auropunctata* (petite fourmi de feu), *Axis axis* (cerf axis, chital), *Pycnonotus cafer* (bulbul à ventre rouge), *Fallopia baldschuanica* (renouée grimpante, renouée de Boukhara), *Phytolacca americana* (phytolaque américaine, raisin d'amérique), *Boccardia proboscidea* (un polychète), *Schizoporella japonica* (un bryozoaire). Les évaluations des risques et les annexes de gestion connexes ont été produites parallèlement aux tâches visant à élaborer et à tenir à jour un modèle d'évaluation des risques et à recueillir des données sur les techniques de gestion, les coûts de mise en œuvre et la rentabilité. Un atelier de deux jours a été organisé pour finaliser les évaluations des risques à la suite d'une revue des analyses examen par des experts pairs. Les évaluations des risques seront utilisées comme éléments probants pour déterminer si l'on doit envisager d'inscrire les espèces cibles sur la liste des espèces exotiques envahissantes préoccupantes pour l'Union en vertu du règlement (UE) n° 1143/2014 concernant la prévention et la gestion de l'introduction et de la propagation d'espèces exotiques envahissantes (Règlement EEE).

Samenvatting: Het beleid en beheer rond invasieve uitheemse soorten (IUS) dient onderbouwd te worden met risicobeoordelingen die gebaseerd zijn op de best beschikbare kennis. In de afgelopen drie jaar werd een sjabloon voor het maken van gestructureerde risicobeoordelingen ontwikkeld. Het werd uitgetest voor dertig geselecteerde soorten uit mariene, terrestrische en zoetwater ecosystemen uit verschillende taxonomische groepen. In dit rapport presenteren we tien risicobeoordelingen en bijbehorende beheerbijlagen voor de IUS die in het afgelopen jaar opgesteld werden. De geselecteerde soorten waren *Channa argus* (noordelijke slangenkopvis), *Ameiurus melas* (zwarte Amerikaanse dwergmeerval), *Ameiurus nebulosus* (bruine Amerikaanse dwergmeerval), *Wasmannia auropunctata* (dwergvuurmier), *Axis axis* (axishert, chital), *Pycnonotus cafer* (roodbuikbulbul), *Fallopia baldschuanica* (Chinese bruidssluier), *Phytolacca americana* (westerse karmozijnbes), *Boccardia proboscidea* (een borstelworm), *Schizoporella japonica* (een mosdiertje). Naast het uitvoeren van de risicoanalyses en het opstellen van de bijlage met beheeropties werd ook het sjabloon voor het uitvoeren van risicoanalyses verder verfijnd, evenals de template om informatie te verzamelen over mogelijke beheermethodes, hun implementatiekosten en effectiviteit. Na peer-review werden de risicoanalyses besproken en afgewerkt tijdens een tweedaagse workshop met auteurs, reviewers en experten. De risicoanalyses zullen worden gebruikt in de besluitvorming rond de opname van soorten op de lijst van voor de Unie zorgwekkende invasieve uitheemse soorten sensu Verordening (EU) 1143/2014 inzake de preventie en het beheer van de introductie en verspreiding van invasieve uitheemse soorten (de IAS-Verordening).

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Annex 5: Risk assessment & annex on measures for *Axis axis* (chital)
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Annex 8: Risk assessment & annex on measures for *Phytolacca americana* (American pokeweed)
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Annex 10: Risk assessment & annex on measures for *Schizoporella japonica* (orange ripple bryozoan)

Contributors

	Allocation of tasks
Centre for Ecology & Hydrology, UK	
Helen Roy	Lead implementation of all tasks, supervision, quality control
Björn Beckmann	Lead implementation of model projections of invasive distributions for all risk assessments
Jodey Peyton	Co-lead of Task 2. Facilitate meetings, workshops, and provide project support
Steph Rorke	Coordination of data across project
Environment Agency Austria, Austria	
Wolfgang Rabitsch	Co-lead Tasks 1 and 5, Support to all tasks
Franz Essl	Support to all tasks providing expertise on terrestrial and freshwater plant species
Stefan Schindler	Support to all tasks and Data Mining expert
IUCN SSC Invasive Species Specialist Group	
Riccardo Scalera	Co-lead of Tasks 1, 3, 4 and 5. Support to all tasks
CABI, Switzerland and UK	
Marc Kenis	Co-lead Task 4. Support to all tasks
Dick Shaw	Co-lead Task 4. Support to all tasks
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Additional experts and affiliations:

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Phytolacca americana (American pokeweed)	Guillaume Fried ANSES, Montpellier, France

Executive Summary

There is an urgent need to provide evidence-based assessments of the risks posed by invasive alien species (IAS) to prioritise action. Risk assessments underpin IAS policies in many ways: informing legislation; providing justification of restrictions in trade or consumer activities; prioritising surveillance and rapid response. The risk assessments carried out in the framework of this study will provide evidence to inform whether the target species should be considered for inclusion on the list of invasive alien species of Union concern under Regulation (EU) 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (the IAS Regulation).

This is the second renewal of the Study Contract No 070202/2016/740982/ETU/ENV.D.2 and 07.0202/2017/763379/ETU/ENV.D2 "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention".

Here we present the outcomes of the study consisting of four tasks:

Task 1: Maintain the template for the risk assessments up to date and fit for purpose on the basis of experience gained
Task 2: Develop the list of species to be assessed
Task 3: Prepare the risk assessments
Task 4: Collect evidence on management techniques, implementation costs and cost-effectiveness

Task 1: The template for the risk assessments was modified from the version used in year 2 to reflect decisions taken during the final workshop of 11-12 October 2018. The updated template, approved with the European Commission (DG Environment), was used for completing all 10 risk assessments under Task 3.

Task 2: The project team was divided into five expert thematic groups: Freshwater animals, Marine species, Plants (including freshwater), Terrestrial invertebrates and Vertebrates. The groups were invited to select the IAS identified as very high or high priority for risk assessment by a previous horizon scanning exercise (final report of the contract ENV.B.2/ETU/2014/00161), supplementing with any emerging IAS and consulting the list developed by Carboneras et al (2017). At the kick-off meeting of 12 December 2018 the European Commission was presented with a draft list of 10 species considered by the project team as potential candidates for risk assessment. The European Commission also presented a list for consideration. Through consultation with the project team and European Commission, 10 species were selected as priority for risk assessment in the framework of this study. Emphasis was placed on IAS that are not yet present in the European Union (or have a limited distribution) and have the potential to have an adverse impact on biodiversity. The ten selected species were:

1. *Channa argus* (northern snakehead)
2. *Ameiurus melas* (black bullhead)
3. *Ameiurus nebulosus* (brown bullhead)
4. *Wasmannia auropunctata* (little fire ant)
5. *Axis axis* (chital)
6. *Pycnonotus cafer* (red-vented bulbul)

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[1] Available at:
http://ec.europa.eu/environment/nature/invasivealien/docs/Prioritising%20prevention%20efforts%20through%20horizon%20scanning.pdf

7. *Fallopia baldschuanica* (Russian vine)
8. *Phytolacca americana* (American pokeweed)
9. *Boccardia proboscidea* (polychaete worm)
10. *Schizoporella japonica* (orange ripple bryozoan)

Task 3: The risk assessments were developed over the entire duration of the contract and involved experts from within the project team, along with additional experts acting as lead authors and / or peer-reviewers). Additionally, for a selection of species, dedicated species distribution models were developed to increase the knowledge base required to strengthen the result of the relevant risk assessments. All risk assessments were subject to peer-review by at least two independent experts. The comments from the reviewers and responses from the risk assessment authors are documented within this report. The risk assessments were completed within a final two-day workshop held on 16-17 October 2019, in which each risk assessment was presented, discussed and amended to ensure overall comprehensiveness and consistency in approaches by assessors.

Task 4: Management annexes were produced for each of the selected species, by the teams producing the risk assessments but also drawing on the expertise of additional specialists. These were based on the available key scientific evidence gathered from a variety of sources with the aim to inform risk management decisions.

In conclusion the risk assessment approach employed is considered comprehensive and robust. No major changes were suggested during the final workshop of 16-17 October 2019.

All work is documented in the final study report, including a number of issues for further consideration and key recommendations. The final study report is accompanied by 10 annexes, one for each of the ten selected species, including the risk assessment and the management annex with information on measures and costs.

Résumé exécutif

Il est urgent de fournir des évaluations des risques posés par les espèces exotiques envahissantes (EEE) qui soient fondées sur des données probantes afin d'établir les priorités d'action. Les évaluations des risques sous-tendent les politiques relatives aux EEE de plusieurs façons : en informant la législation, en justifiant les restrictions dans les activités commerciales ou de consommation, en donnant la priorité à la surveillance et à la réponse rapide. Les évaluations des risques réalisées dans le cadre de cette étude fourniront des éléments permettant de déterminer si on doit envisager d'inscrire l'espèce cible sur la liste des espèces exotiques envahissantes préoccupantes pour l'Union en vertu du Règlement (UE) 1143/2014 sur la prévention et la gestion de l'introduction et de la propagation des espèces exotiques envahissantes (le règlement EEE).

Il s'agit du deuxième renouvellement du contrat d'étude n° 070202/2016/740982/ETU/ENV.D.2 et 07.0202/2017/763379/ETU/ENV.D2[2] "Étude sur les espèces exotiques envahissantes - Élaboration d'évaluations des risques pour lutter contre les espèces prioritaires et renforcer la prévention".

Nous présentons ici les résultats de l'étude, qui comprend quatre tâches :

Tâche 1 : Tenir à jour le modèle d'évaluation des risques et l'adapter aux besoins sur base de l'expérience acquise
Tâche 2 : Élaborer la liste des espèces à évaluer
Tâche 3 : Préparer les évaluations des risques
Tâche 4 : Recueillir des données sur les techniques de gestion, les coûts de mise en œuvre et la rentabilité

Tâche 1 : Le modèle pour les évaluations des risques a été modifié par rapport à la version utilisée au cours de l'année 2 afin de refléter les décisions prises lors de l'atelier final des 11 et 12 octobre 2018. Le modèle mis à jour, approuvé avec la Commission Européenne (DG Environnement), a été utilisé pour réaliser les 10 évaluations des risques dans le cadre de la Tâche 3.

Tâche 2 : L'équipe du projet a été divisée en cinq groupes thématiques d'experts : Animaux d'eau douce, Espèces marines, Plantes (y compris d'eau douce), Invertébrés terrestres et Vertébrés. Les groupes ont été invités à sélectionner les EEE identifiées comme très prioritaires ou hautement prioritaires pour l'évaluation des risques lors d'un exercice précédent d'horizon scanning (rapport final du contrat ENV.B.2/ETU/2014/0016), en complétant par des EEE émergentes et en consultant la liste élaborée par Carboneras et al (2017). Lors de la réunion de lancement du 12 décembre 2018, la Commission Européenne a reçu un projet de liste de 10 espèces considérées par l'équipe de projet comme des candidats potentiels à l'évaluation des risques. La Commission Européenne a également présenté une liste pour examen. En consultation avec l'équipe de projet et la Commission Européenne, 10 espèces ont été sélectionnées comme prioritaires pour l'évaluation des risques dans le cadre de cette étude. L'accent a été mis sur les EEE qui ne sont pas encore présentes dans l'Union Européenne (ou qui ont une distribution limitée) et qui pourraient avoir un impact négatif sur la biodiversité. Les dix espèces sélectionnées sont :

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[2] Disponible sur:
http://ec.europa.eu/environment/nature/invasivealien/docs/Prioritising%20prevention%20efforts%20through%20horizon%20scanning.pdf

1. *Channa argus* (poisson à tête de serpent)
2. *Ameiurus melas* (poisson-chat)
3. *Ameiurus nebulosus* (barbotte brune)
4. *Wasmannia auropunctata* (petite fourmi de feu)
5. *Axis axis* (cerf axis, chital)
6. *Pycnonotus cafer* (bulbul à ventre rouge)
7. *Fallopia baldschuanica* (renouée grimpante, renouée de Boukhara)
8. *Phytolacca americana* (phytolaque américaine, raisin d'amérique)
9. *Boccardia proboscidea* (un polychète)
10. *Schizoporella japonica* (un bryozoaire)

Tâche 3 : Les évaluations des risques ont été élaborées pendant toute la durée du contrat et ont fait appel à des experts de l'équipe de projet, ainsi qu'à d'autres experts agissant comme auteurs principaux et/ou évaluateurs). De plus, pour une sélection d'espèces, des modèles de distribution des espèces ont été développés afin d'augmenter la base de connaissances requise pour renforcer de manière pertinente les résultats des évaluations de risques. Toutes les évaluations des risques ont été soumises à un examen par les pairs par au moins deux experts indépendants. Les commentaires des examinateurs et les réponses des auteurs des évaluations des risques sont documentés dans le présent rapport. Les évaluations des risques ont été réalisées dans le cadre d'un dernier atelier de deux jours tenu les 16 et 17 Octobre 2019, au cours duquel chaque évaluation des risques a été présentée, discutée et modifiée afin d'assurer l'exhaustivité et la cohérence globales des approches des évaluateurs.

Preamble

The work reported here was done in line with the Original Technical Proposal tendered to the European Commission for this contract. This work was discussed at a kick-off meeting, which resulted in no changes to the overall work plan. This final report summarises the project. The ten completed risk assessments are in annexes linked to this report.

Overview of Tasks

Task 1 Maintain the template for the risk assessments up to date and fit for purpose on the basis of experience gained

Leading experts: Wolfgang Rabitsch (EAA), Riccardo Scalera (ISSG)

Other contributors: Etienne Branquart (EPPO), Helen Roy (CEH), Rob Tanner (EPPO), Sven Bacher (University of Fribourg)

At the beginning of this project, the European Commission (hereafter EC) provided a template for the risk assessments as a guide reflecting all elements required by Article 5(1) of Regulation (EU) 1143/2014 on invasive alien species[3] (hereafter the IAS Regulation).

During the first year of this contract (07.0202/2016/740982/ETU/ENV.D2) the content and text of the template were incorporated into the Risk Assessment Scheme developed by the GB Non-Native Species Secretariat (GB Non-Native Risk Assessment - GBNNRA) to ensure full compliance with the requirements of Article 5(1) of the IAS Regulation. Assessors were provided with this document including the explanatory text within the document, although indicated in a different colour. This explanatory text was deleted in the final version of the completed risk assessments.

The template was adapted according to the requirements of the Delegated Regulation (EU) 2018/968 of 30 April 2018 supplementing Regulation (EU) 1143/2014 and further discussions with the assessors, the peer-reviewers, the European Commission and discussions at the final workshops of the first and second years of the contract in 2017 and 2018.

The risk assessment template used in the framework of this study is provided on page 33 onwards of this report.

Task 2 Develop the list of species to be assessed

Leading experts: Jodey Peyton (CEH) and Alan Stewart (University of Sussex)

Other contributors: Argyro Zenetos (HCMR), Elena Tricarico (University of Florence), Jørgen Eilenberg (University of Copenhagen), Jack Sewell (MBA), Wolfgang Rabitsch, Franz Essl (EAA), Frances Lucy (Institute of Technology), Rob Tanner (EPPO), Tim

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[3] Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species: http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1513584398867&uri=CELEX:32014R1143

Adriaens (RINF), Sonia Vanderhoeven (Belgian Biodiversity Platform), Sven Bacher (University of Fribourg).

Documentation of the process of species selection

The objective of Task 2 was to select a list of ten priority species, supported by the full documentation of the process of species selection, for subsequent risk assessment in Task 3.

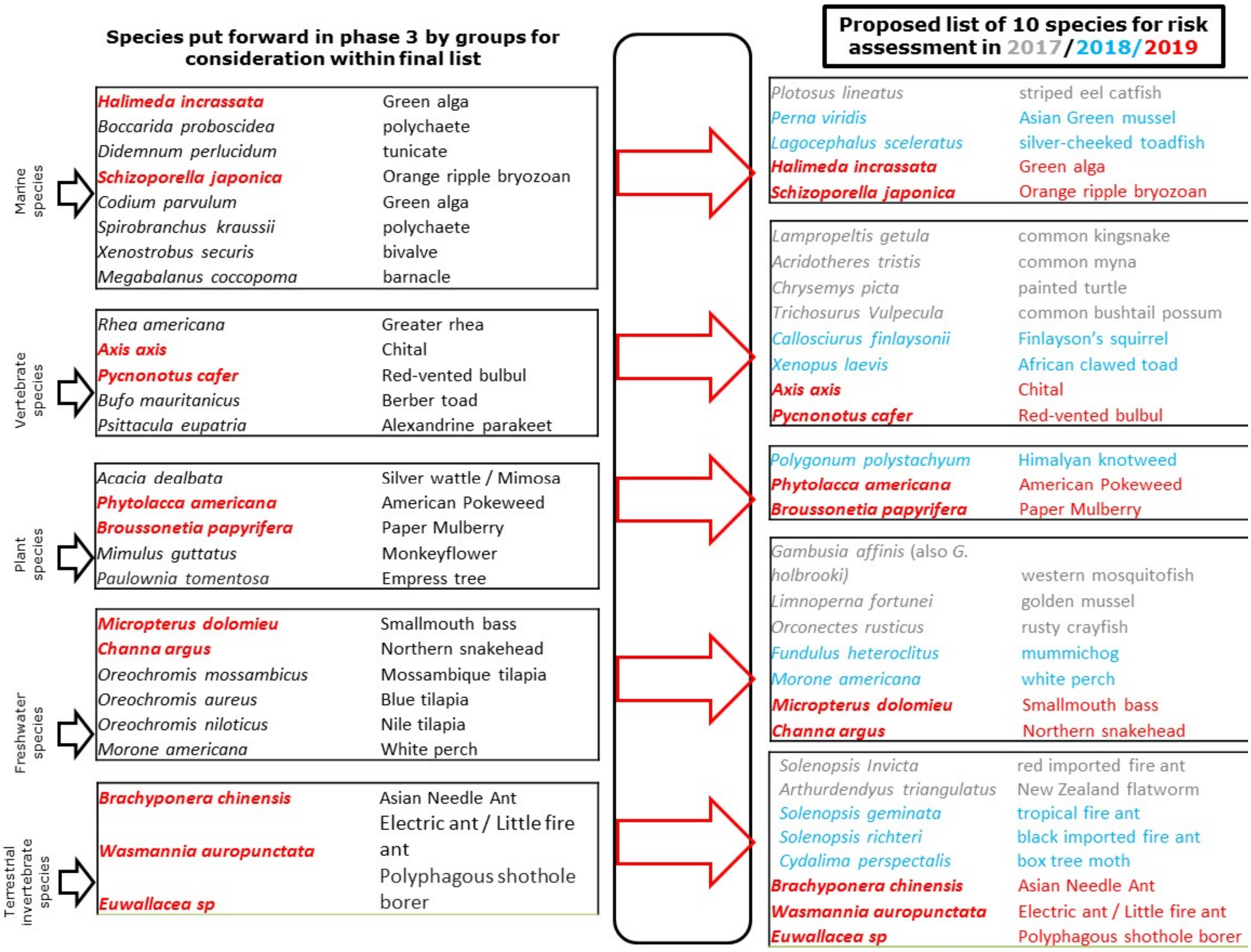
As in previous exercises, groups of experts were convened with expertise in five taxonomic/ecological groupings. The membership of these groups was as follows (group co-leaders in bold):

Freshwater animals: **Frances Lucy**, **Elena Tricarico**, Hugo Verreycken, Gordon Copp, Paul Stebbing
Marine species: **Argyro Zenetos**, **Jack Sewell**
Plants (including freshwater): **Rob Tanner**, Oli Pescott, Dan Chapman, Franz Essl, Etienne Branquart, Sonia Vanderhoeven
Terrestrial invertebrates: **Marc Kenis**, **Dick Shaw**, Karsten Schonrogge, Wolfgang Rabitsch, Alan Stewart, Jorgen Eilenberg
Vertebrates: **Riccardo Scalera**, **Tim Adriaens**, Wolfgang Rabitsch, Piero Genovesi, Pete Robertson, Niall Moore, Olaf Booy, Sven Bacher, Yasmine Verzelen

Group co-leaders were responsible for consulting their expert group members (by email, Skype, teleconference etc.) to request reasoned evidence-based suggestions for species to be included in the list for risk assessment. The procedure adopted was as documented in the Final Report of study 07.0202/2018/763379/ETU/ENV.D2. A list of species was presented to the European Commission (Table 1; Figure 1).

Table 1: List of species identified by the expert subgroups for consideration by the project team and European Commission for risk assessment in 2019 (see Figure 2; Table 2).

Rank from EU Horizon Scanning (Roy et al. 2015)	Expert subgroup	Species	Common Name
VERY HIGH	Freshwater	*Micropterus dolomieu*	Smallmouth bass
VERY HIGH	Freshwater	*Channa argus*	Northern snakehead
HIGH	Terrestrial invertebrates	*Brachyponera chinensis*	Asian Needle Ant
	Terrestrial invertebrates	*Wasmannia auropunctata*	Electric ant / Little fire ant
	Terrestrial invertebrates	*Euwallacea sp*	Polyphagous shothole borer
HIGH	Vertebrates	*Axis axis*	Chital
VERY HIGH	Vertebrates	*Pycnonotus cafer*	Red-vented bulbul
VERY HIGH	Plants	*Phytolacca americana*	American Pokeweed
VERY HIGH	Plants	*Broussonetia papyrifera*	Paper Mulberry
	Marine	*Halimeda incrassata*	Green alga
	Marine	*Schizoporella japonica*	Orange ripple bryozoan



Species put forward in phase 3 by groups for consideration within final list

Marine species

Halimeda incrassata	Green alga
Boccarida proboscidea	polychaete
Didemnum perlucidum	tunicate
Schizoporella japonica	Orange ripple bryozoan
Codium parvulum	Green alga
Spirobranchus kraussii	polychaete
Xenostrobus securis	bivalve
Megabalanus coccopoma	barnacle

Vertebrate species

Rhea americana	Greater rhea
Axis axis	Chital
Pycnonotus cafer	Red-vented bulbul
Bufo mauritanicus	Berber toad
Psittacula eupatria	Alexandrine parakeet

Plant species

Acacia dealbata	Silver wattle / Mimosa
Phytolacca americana	American Pokeweed
Broussonetia papyrifera	Paper Mulberry
Mimulus guttatus	Monkeyflower
Paulownia tomentosa	Empress tree

Freshwater species

Micropterus dolomieu	Smallmouth bass
Channa argus	Northern snakehead
Oreochromis mossambicus	Mossambique tilapia
Oreochromis aureus	Blue tilapia
Oreochromis niloticus	Nile tilapia
Morone americana	White perch

Terrestrial invertebrate species

Brachyponera chinensis	Asian Needle Ant
Wasmannia auropunctata	Electric ant / Little fire ant
Euwallacea sp	Polyphagous shothole borer

Proposed list of 10 species for risk assessment in 2017/2018/2019

Plotosus lineatus	striped eel catfish
Perna viridis	Asian Green mussel
Lagocephalus sceleratus	silver-cheeked toadfish
Halimeda incrassata	Green alga
Schizoporella japonica	Orange ripple bryozoan

Lampropeltis getula	common kingsnake
Acridotheres tristis	common myna
Chrysemys picta	painted turtle
Trichosurus Vulpecula	common bushtail possum
Callosciurus finlaysonii	Finlayson's squirrel
Xenopus laevis	African clawed toad
Axis axis	Chital
Pycnonotus cafer	Red-vented bulbul

Polygonum polystachyum	Himalyan knotweed
Phytolacca americana	American Pokeweed
Broussonetia papyrifera	Paper Mulberry

Gambusia affinis (also *G. holbrooki*)	western mosquitofish
Limnoperna fortunei	golden mussel
Orconectes rusticus	rusty crayfish
Fundulus heteroclitus	mummichog
Morone americana	white perch
Micropterus dolomieu	Smallmouth bass
Channa argus	Northern snakehead

Solenopsis Invicta	red imported fire ant
Arthurdendyus triangulatus	New Zealand flatworm
Solenopsis geminata	tropical fire ant
Solenopsis richteri	black imported fire ant
Cydalima perspectalis	box tree moth
Brachyponera chinensis	Asian Needle Ant
Wasmannia auropunctata	Electric ant / Little fire ant
Euwallacea sp	Polyphagous shothole borer

Figure 1. Process of selection of species for risk assessment including updates for 2018/2019.

Discussion of some issues arising during the kick-off meeting

The species lists (Table 1) was discussed during the kick-off meeting and the European Commission provided broad reflections including:

1. Existing risk assessments for completion
   There are a number of risk assessments that are at various stages of development and with varying degrees of alignment with the minimum standards (Roy *et al.* 2018) namely:

Celastrus orbiculatus
https://www.nvwa.nl/documenten/plant/planten-in-de-natuur/exoten/risicobeoordelingen/risicoanalyserapport-boomwurger
Micropterus dolomieu
https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/risicoanalyserapport-zwartbaars

2. Recent horizon scanning exercise for marine species https://easin.jrc.ec.europa.eu/easin/NewsAndEvents/DetailEvents/c1f4939e-2a09-4095-b3cf-5c91b65834ee.
   Recognising the following risk assessments are complete or in late stages of preparation:

Plotosus lineatus: Lessepsian species, escape from confinement
Limnoperna fortunei: angling/fishing equipment, hull fouling
Perna viridis: ballastwater, hull fouling, release in nature for fishing
Morone americana: ballastwater, contaminant in aquaculture, release in nature for fishing
Lagocephalus sceleratus: Lessepsian species, escape from confinement
Rapana venosa: hull fouling, ballastwater
Hemigrapsus sanguineus: ballastwater, hull fouling

Therefore, the Commission invited the project team to consider the following species: *Channa argus*, *Ameiurus melas*, *Ameiurus nebulosus*, *Fallopia japonica*, *Fallopia sachalinensis*, *Fallopia* × *bohemica*, *Fallopia baldschuanica*, *Pterois miles*, *Celastrus orbiculatus* alongside consideration of ballast water and Lessepsian species.

Through extensive consultation with the project team and further feedback from the Commission a list of 11 species (Table 2) was agreed for risk assessment.

Table 2: List of species identified by the expert subgroups and agreed by the European Commission for risk assessment in 2019 (see Figure 1; Table 1). Notes: Micropterus dolomieu (by modifying the Dutch PRA: https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/risicoanalyserapport-zwartbaars); Celastrus orbiculatus (by modifying Dutch PRA: https://upload.eppo.int/download/412of22fb570b

Rank from EU Horizon Scanning (Roy et al. 2015)	Expert subgroup	Species	Common Name	Notes
VERY HIGH	Freshwater	*Micropterus dolomieu*	Smallmouth bass	Modify Dutch RA
VERY HIGH	Freshwater	*Channa argus*	Northern snakehead	Modify Spanish draft
	Freshwater	*Ameiurus melas*	Black bullhead	Modify Spanish draft
	Freshwater	*Ameiurus nebulosus*	Brown bullhead	Modify Spanish draft
	Terrestrial invertebrates	*Wasmannia auropunctata*	Electric ant / Little fire ant	
HIGH	Vertebrates	*Axis axis*	Chital	
VERY HIGH	Vertebrates	*Pycnonotus cafer*	Red-vented bulbul	
	Plants	*Fallopia baldschuanica*	Russian Vine	
VERY HIGH	Plants	*Celastrus orbiculatus*	Oriental bittersweet	Modify Dutch RA
	Marine	*Boccardia proboscidea*	A polychaete worm	
	Marine	*Schizoporella japonica*	Orange ripple bryozoan	

Post meeting discussions with the EC

Two of the risk assessments already in preparation were reviewed by the EC following the kick-off meeting:

PLANTS: Celastrus orbiculatus (Dutch PRA: https://upload.eppo.int/download/412of22fb570b)
FRESHWATER: Micropterus dolomieu (Dutch PRA: https://www.nvwa.nl/documenten/dier/dieren-in-de-natuur/exoten/risicobeoordelingen/risicoanalyserapport-zwartbaars

It was agreed that these should not be included within this study but instead an additional species *Phytolacca americana* will be assessed.

Therefore, the final list is shown in Table 3.

Table 3: List of species identified by the expert subgroups and agreed by the European Commission for risk assessment in 2019 (see Figure 1; Table 1 and Table 2).

Rank from EU Horizon Scanning (Roy et al. 2015)	Expert subgroup	Species	Common Name	Notes
VERY HIGH	Freshwater	*Channa argus*	Northern snakehead	Modify Spanish draft
	Freshwater	*Ameiurus melas*	Black bullhead	Modify Spanish draft
	Freshwater	*Ameiurus nebulosus*	Brown bullhead	Modify Spanish draft
	Terrestrial invertebrates	*Wasmannia auropunctata*	Electric ant / Little fire ant	
HIGH	Vertebrates	*Axis axis*	Chital	
VERY HIGH	Vertebrates	*Pycnonotus cafer*	Red-vented bulbul	
	Plants	*Fallopia baldschuanica*	Russian Vine	
VERY HIGH	Plants	*Phytolacca americana*	American pokeweed	
	Marine	*Boccardia proboscidea*	A polychaete worm	
	Marine	*Schizoporella japonica*	Orange ripple bryozoan	

In summary, final selection of the ten species was made through repeated email and teleconference discussions between the Task 2 leaders informed by consultations with the group co-leaders and European Commission. All views and concerns were given full consideration with further information or opinion being sought from members of the expert teams where necessary. The final list is the result of a consensus between all group co-leaders and discussions with the European Commission. Of particular note were reflections on the outputs from the recent horizon scanning exercise for marine species https://easin.jrc.ec.europa.eu/easin/NewsAndEvents/DetailEvents/c1f4939e-2a09-4095-b3cf-5c91b65834ee and also consideration of risk assessments under development by others for example *Channa argus* (northern snakehead), *Ameiurus* *melas* (black bullhead) and *Ameiurus nebulosus* (brown bullhead) for which drafts had been submitted to the European Commission.

Subjectivity of approach:

The subjectivity of the overall approach should be noted. Our previous horizon scanning exercise showed that it was difficult to identify an objective and reliable method to prioritise species, particularly across taxonomic/ecological groups, with a greater degree of precision than by using the comparatively crude assignment of species to 'very high', 'high' or 'medium' risk categories. For transparency, we have included in the horizon scanning report the detailed scores in the species table, but we emphasise that these should be treated with caution due to the difficulty in moderating scores between the taxonomic groups.

Task 3 Prepare the risk assessments

Leading experts: Riccardo Scalera (IUCN ISSG), Rob Tanner (EPPO), Oli Pescott (CEH), Björn Beckmann (CEH), Beth Purse (CEH)

Other contributors: Helen Roy (CEH), Gordon Copp (Cefas), Wolfgang Rabitsch (EAA), Jørgen Eilenberg (University of Copenhagen), Frances Lucy (Institute of Technology), Tim Adriaens (INBO), Argyro Zenetos (HCMR), Jack Sewell (MBA), Marc Kenis (CABI), plus other relevant experts (Table 4).

Table 4. List of ten species for risk assessment and names of contributors to the process

Species	Lead authors of risk assessment	Experts for peer-reviewing	Management expert
Channa argus (northern snakehead)	Hugo Verreycken Luke Aislabie Gordon Copp	Elena Tricarico Frances Lucy	Hugo Verreycken Luke Aislabie Gordon Copp
Ameiurus melas (black bullhead)	Hugo Verreycken Luke Aislabie Gordon Copp	Elena Tricarico Frances Lucy	Hugo Verreycken Luke Aislabie Gordon Copp
Ameiurus nebulosus (brown bullhead)	Hugo Verreycken Luke Aislabie Gordon Copp	Elena Tricarico Frances Lucy	Hugo Verreycken Luke Aislabie Gordon Copp
Wasmannia auropunctata (little fire ant)	Olivier Blight	Richard Shaw Marc Kenis Alan Stewart	Olivier Blight Peter Robertson
Axis axis (chital)	Riccardo Scalera Wolfgang Rabitsch Piero Genovesi Sven Bacher	Wojciech Solarz Anonymous reviewer	Riccardo Scalera Peter Robertson

	Tim Adriaens Yasmine Verzelen Peter Robertson Björn Beckmann		
Pycnonotus cafer (red-vented bulbul)	Yasmine Verzelen Tim Adriaens Riccardo Scalera Björn Beckmann Martin Thibault Peter Robertson Marianne Kettunen Sven Bacher Wolfgang Rabitsch	Tom Evans Jorgen Eilenberg	Yasmine Verzelen Peter Robertson
Fallopia baldschuanica (Russian vine)	Rob Tanner Richard Shaw Johan van Valkenburg	Giuseppe Brundu Etienne Branquart	Rob Tanner Peter Robertson
Phytolacca americana (American pokeweed)	Rob Tanner Guillaume Fried	Johan van Valkenburg Giuseppe Brundu	Rob Tanner Peter Robertson
Boccardia proboscidea (polychaete worm)	Marika Galanidi Argyro Zenetos Björn Beckmann	Vasily Radashevsky Jack Sewell	Marika Galanidi Argyro Zenetos Peter Robertson
Schizoporella japonica (orange ripple bryozoan)	Jack Sewell Christine Wood	Marika Galanidi Argyro Zenetos	Jack Sewell Christine Wood Peter Robertson

Specific inputs for a number of species to be risk assessed were also provided by EAZA[4] and EPO[5], through the European Commission, and were used as supporting information for the pathways related to the zoo/aquaria and the pet trade sectors, respectively.

Selection of species for model and summary of modelling approach

Species Distribution Models (SDMs) were created for six species, for which the risk assessment teams considered a model to be an important assessment aid: This was particularly for species where no recent models and / or models with a relevant geographical scope already existed, and for which sufficient information in terms of distribution records and ecophysiological data was available. The species for which SDMs were constructed were: *Fallopia baldschuanica*, *Phytolacca americana*, *Boccardia proboscidea*, *Wasmannia auropunctata*, *Pycnonotus cafer* and *Axis axis*.
Species distribution records were obtained from nine large online databases – the Global Biodiversity Information Facility (GBIF), the Biodiversity Information Serving Our Nation database (BISON), iNaturalist, eBird, the Berkeley Ecoinformatics Engine database, the VertNet databases, the Integrated Digitized Biocollections (iDigBio), the Ocean Biogeographic Information System (OBIS), and the Atlas of Living Australia. Additional

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[4] European Association of Zoos and Aquaria

[5] The European Pet Organization

records were supplied for several species by the respective risk assessment team. Records were scrutinised and any dubious ones removed (e.g. captive records), as were those where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid).

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1. These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman *et al.* 2019). Therefore, the background sampling region included:

- The area accessible by native populations, in which the species is likely to have had sufficient time to disperse to all locations; AND
- A buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints.

Within the background region, 10 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort; we used the density of records held by GBIF for the higher taxonomic group of each species as a proxy for recording effort.

Climate data for modelling terrestrial species were selected from the 'Bioclim' variables contained within the WorldClim database, and for marine species from the 'Bio-ORACLE' set of GIS rasters providing geophysical, biotic and environmental data for surface and benthic marine realms (Tyberghein *et al.* 2012; Assis *et al.* 2018). For species where climatic moisture availability was considered important, a Climatic moisture index (CMI) was calculated as the ratio of mean annual precipitation to potential evapotranspiration by estimating monthly potential evapotranspirations from the WorldClim monthly temperature data and solar radiation using the simple method (Zomer *et al.* 2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively.

Additional non-climatic predictor layers were used for several species where these were considered relevant: Tree cover, estimated from the MODerate-resolution Imaging Spectroradiometer (MODIS) satellite continuous tree cover raster product, produced by the Global Land Cover Facility (http://glcf.umd.edu/data/vcf/), and a Human influence index (HII), since many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society & Center for International Earth Science Information Network, Columbia University, 2005). This is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers).

Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings (Generalised linear model (GLM), Generalised boosting model (GBM), Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline, Artificial neural network (ANN), Multivariate adaptive regression splines (MARS), Random forest (RF), and Maxent). Since background samples tended to be larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures: AUC, the area under the receiver operating characteristic curve, Cohen's Kappa, and TSS, the true skill statistic. Ensemble models were created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. The projections were then classified into suitable and unsuitable regions using the 'minROCdist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe (Elith, Kearney & Phillips 2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Finally, we calculated the percentage area of each EU member state, and of each Biogeographical region of Europe (Bundesamt für Naturschutz (BfN), 2003), projected to be suitable for establishment of each terrestrial species, both for the current climate and for projected climate for the 2070s under the two climate change scenarios scenarios mentioned above. For marine species, we calculated percentage areas projected to be suitable of the 12-nautical-mile national waters of European Union countries, and of the marine subregions of Europe (https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf/at_download/file).

Specific comments from lead experts on the risk assessments

All risk assessments and annexes on management were peer reviewed. The comments of the peer reviewers have been taken into consideration as appropriate. The final workshop provided an excellent opportunity to discuss and agree changes and as such, the final versions of the risk assessments and annexes on management represent the consensus reached between authors and peer reviewers. Summary of key points in response to the peer-review:

Channa argus (northern snakehead)

The conclusion of the risk assessment for *Channa argus* is "high overall risk with medium confidence". Although the likelihood of introduction of this species into the risk assessment area is considered unlikely, if this species was to be introduced into the EU, then it is likely to spread and exert major impacts. It was concluded that the internet trade cannot be dismissed as an active pathway for *Channa argus* and this has been included in the final risk assessment.

Ameiurus melas (black bullhead)
Ameiurus nebulosus (brown bullhead)

Although within the same genus, *Ameiurus melas* and *Ameiurus nebulosus* evidence for the development of the risk assessments was collected through specific literature searches for each species. The conclusion of both risk assessments for *A. melas* and *A. nebulosus* is "moderate overall risk with medium confidence".

The distribution of both species includes several EU countries, but populations are localized. There is one report of *A. melas* declining (River Po, Italy), while for *A. nebulosus* there is some evidence of decline of the species across its EU range.

Potential impacts of both species include increased turbidity, especially in smaller water bodies and potential decreases in the ecosystem services (mainly angling). There is some concern over *A. melas* presence in national parks and nature reserves (especially in Mediterranean areas), though studies of economic loss produced by *A. melas* are lacking. Other potential impacts include the transmission of fish diseases to some fish species native to most of the EU (e.g. European catfish *Silurus glanis*).

***Wasmannia auropunctata* (little fire ant)**

The conclusion of the risk assessment for *Wasmannia auropunctata* is "high overall risk with medium confidence". Main issues at the peer review process were:

Confidence scores were adjusted based on expert knowledge and when comparisons were made with other taxa and likelihood scores of 3.9, 3.10,3.11 were elevated from likely to very likely.

The pathway Transport/stowaway (hitchikers in or on airplane) was expanded to cover any journey that does not exceed a few days to include all means but exclude shipping freight. Two additional pathways were also added namely: Transportation of habitat material (soil, vegetation, wood, …) and Food contaminant (including of live food). Further evidence was found to estimate the volume of trade in plants-for-planting and this was reported within the nursery trade transport contaminant pathway section.

In terms of impacts, Q5.14 caused some debate as to whether causing blindness in humans and pets could constitute a major or moderate impact and it was felt that even at a limited scale this should be considered a major impact.

***Axis axis* (chital)**

The conclusion of the risk assessment for *Axis axis* is "moderate overall risk with medium confidence". Climate change may increase impacts by increasing the amount of suitable habitat. Main issues at the peer review process were:

One reviewer (requested to remain anonymous) commented extensively throughout the text. This reviewer recognized that it is extremely difficult to prepare a risk assessment for a species which occurs within the EU but only in the wild (feral) in offshore islands of Croatia. It is therefore necessary to generalise from the published experience of other species. However, the reviewer also cautioned against placing too much emphasis on general information from regions beyond the study area. According to the peer-reviewer far too much reliance was placed on the work of from North America - with scant regard to more relevant European literature on (generic) deer impacts. Additionally the peer-reviewer also considered that there was too much reliance on literature exploring effects of overabundance, whereas the reviewer considered it would have been more valuable to consider an exploration of impacts at more "typical" density. Also, in the view of this peer-reviewer, the most likely source of entry into the EU is by escape of population nuclei from deer parks or private collections. In relation to this, the authors pointed out that the reason why the information discussed on the risk assessment is mostly based specifically on Axis deer only (despite the paucity of relevant data) is because it was deemed inappropriate (and too speculative) to base any assessment on evidence from other deer with a different ecology and natural history. The same approach was previously confirmed during the assessment of *Chrysemis picta*, for which it was not consider appropriate to base the discussion on data from similar species, e.g. *Trachemys scripta*. The risk is that any statement could be questioned, if based on other taxa with a different ecology and natural history. For this reason, the authors deemed it sufficient to bring some examples of deer in general to only a few situations, for example when dealing with the impact of deer due to overabundance. Given the paucity of data on Axis deer, the authors based the analysis on a few generic papers only because they considered it unnecessary to make an exhaustive review on the issue based on other deer species only. Regarding pathways, deer parks and private collections are indeed considered as key pathways, despite the paucity of specific data on escapes, and all available/necessary information was discussed in the relevant sections.

The second peer-reviewer had many comments but these were minor. In general, most of the comments focussed on clarifying and fine-tuning the scores and the level of confidence, with the aim of ensuring consistency throughout the document. Some inconsistency was noted in relation to the scores (i.e. likelihood) of other protocols (i.e. Harmonia+) and relevant thresholds, which in fact may create confusion. The interpretation of some questions, particularly in relation to differences between the likelihood of introduction and entry, required some extensive discussion.

Pycnonotus cafer (red-vented bulbul)

The conclusion of the risk assessment for *Pycnonotus cafer* is "moderate overall risk with medium confidence". Main issues at the peer review process were:

The results of the modelling were discussed and it was noted that it is counterintuitive that Cyprus is deemed suitable for establishment but Malta is not (whereas average yearly temperature only differs by 1°C). This has to do with the absence of climate data for a small island like Malta. The outputs of the SDM will be adapted accordingly. Additionally the white areas on the map could be considered as either unsuitable areas for establishment or areas with lack of data. The maps were adapted so this distinction is visible. It was also decided at the workshop to additionally include a map showing uncertainty (STDEV) for Europe on top of the global map. There was some discussion on whether or not to use the maps as figures in the risk assessment. This could make the document more engaging, but has the disadvantage of the figures being considered without the complete modelling context (procedure, uncertainty, limitations and assumptions).

A more data-driven approach towards identifying sensitive receptors (native species, habitats and protected areas), using the "endangered area" (i.e. the area suitable for establishment of the species) could be of help to the experts for future risk assessments.

There were some specific points raised on the content:

- Qu. 3.4 comment about cumulative impact of both bulbul species around Valencia was added

- Qu. 3.5 added more information on bird parasites and diseases of RVB: avian malaria and ticks, scores remained the same however reference was made to Grewal (1964)
- Qu. 3.10 (adaptability of the species), it was noted that the species could profit from availability of exotic plants that are not exploited by native passerines.
- Qu. 3.13 statements on the advantage of this species within urban habitats due to urban heat effect and supplemental feeding was removed because it was considered too speculative
- Qu. 3.13 more information was added on suitable areas and limiting factors for establishment
- Qu. 5.3 (potential future impact of the organism on biodiversity): potential impact on native biodiversity was discussed at the workshop in an attempt to identify sensitive receptors (species, protected areas, habitats). As a consequence of reviewer comments and discussions held, more information was provided on potential impacts of this species at all levels of organisation in the risk assessment area, following the same sub-headers of the impact outside the risk assessment area:
    - A section was added on the potential of the species to spread invasive plants, indicating invasive weeds and problem plants and shrubs with fleshy fruits in areas where the species could potentially establish and providing references on this.
    - More examples of bird species which the red-vented bulbul could compete with were added, including some protected species in the risk assessment area.
    - A section was added on potential impact through predation with examples of reptiles and insects that could potentially be impacted in the risk assessment area and relevant references were added.
- Qu. 5.4. (decline in conservation value): a score of minimal did not seem appropriate in response to workshop comments and so the score was changed to minor but with low confidence.

Fallopia baldschuanica **(Russian vine)**

The conclusion of the risk assessment for *Fallopia baldschuanica* is "low overall risk with low confidence". Main issues raised at the workshop and the peer review process were:

It was noted by the participants of the workshop that there may be the potential for hybridization with other *Fallopia* species, similar to the situation seen with *F. japonica* and *F. baldschuanica* which results in the hybrid x *Reyllopia conollyana*. This was added in the risk assessment.

The pathway section was altered to reflect the potential for the dumping of the species into the natural environment as a discard of garden waste and/or waste from ornamental purposes other than horticulture.

It was highlighted that the known impact of the organism on biodiversity at all levels of organisation (question 5.2) was only rated as minor and information in other sections of the risk assessment could be included in section 5.2 to support a moderate rating. The rating for question 5.3 (How important is the potential future impact of the organism on biodiversity at all levels of organization likely to be in the risk assessment area?) was also raised from minor to moderate. The overall rating for entry was raised from moderately likely to likely and the corresponding confidence was raised from low to medium.

Phytolacca americana (American pokeweed)

The conclusion of the risk assessment for *Phytolacca americana* is "moderate overall risk with medium confidence". Main issues raised at the workshop and the peer review process were:

It was noted by the participants of the workshop that *Phytolacca acinosa* is an increasing invasive species in the EU and thus, it could be interesting to conduct a risk assessment for this species in the future.

Some of the workshop participants highlighted that there is a recent report of concerns of increasing observations of *Phytolacca* species in Belgium and this was added to the risk assessment.

It was highlighted that the impact scores seemed a little low based on the evidence detailed in the risk assessment. However, following a detailed evaluation of the scores by the plant group, it was considered that the scores should stay mainly the same as the evidence was not available to increase the score for the species from moderate to major. There was no evidence to support a major score (long-term irreversible ecosystem change, spreading beyond local area).

The authors did however, increase the score for Qu. 5.1. 'How important is the impact of the organism on biodiversity at all levels of organization caused by the organism in its non-native range excluding the risk assessment area?' from minor to moderate.

Boccardia proboscidea (polychaete worm)

The conclusion of the risk assessment for *Boccardia proboscidea* is "high overall risk with medium confidence". Main issues raised at the workshop and the peer review process were:

The external reviewer, a taxonomic expert on the particular polychaete family, provided additional information on the presence of the species with abundant populations in an additional area, not documented in the literature. The reviewer also offered his insights into the potential for establishment in the Black Sea, which corroborated the results of the habitat suitability model.

The most important points raised and discussed during the workshop by the second reviewer were:

- remove the pathway TRANSPORT-STOWAWAY (Bilge waters) from the potential pathways of Introduction and Entry, as it is concluded that it is very unlikely to result in the introduction and entry of *B. proboscidea* propagules in the risk assessment area
- clarify text, where appropriate, to reflect better the differences between the risk of Introduction and the risk of Entry
- be more specific about the way existing management practices may affect the species' ability to survive during transport and storage along the pathway

- offer more information on the potential impacts of the species on hard substrates, their habitats and associated communities, particularly sensitive habitats such as chalk cliffs, and consider raising the overall impact score to major to better reflect such irreversible impacts.

Discussions on the parameters used for the modelling of the species habitat suitability led to a final run of the model, which were incorporated into the submitted version of the risk assessment.

Finally, with respect to the Management Annex, the reviewer's comments prompted some additional statements to describe the potential effectiveness of some of the measures proposed and to justify the scoring of uncertainty.

Schizoporella japonica **(orange ripple bryozoan)**

The conclusion of the risk assessment for *Schizoporella japonica* is "high overall risk with medium confidence". Main issues raised at the workshop and the peer review process were:

Additional notes were added on use of eDNA in detecting colonies.

The references to the link between the presence of multiple ovicells and fecundity has been removed from most sections as it was decided that the statement was based on suppositions published in reviewed literature without any supporting hard evidence.

Some updates on the status of the species populations in Plymouth based on author observations (unpublished) were added.

Information was included about the risk of spread and reduced efficacy of control due to the potential of fragmentation caused by removal attempts.

Amended minimum known temperature and reviewed potential future climate change predictions based on reduced salinity on Iberian coast, SST predicted temperature changes (did not impact predicted range), predicted range based on lower salinity areas in Mediterranean. Some amendments were made to the text, which reflect uncertainty over known tolerances and potential for the species to adapt to extreme environmental conditions.

Workshop to review and finalise risk assessments

A two-day final workshop was organized to enable the project team to come together with the peer-reviewers and other experts to discuss and finalise the text of the risk assessments collaboratively. For this purpose the peer-review process was undertaken (and the required reviews completed) in advance of the workshop. This allowed the discussion of the risk assessments at a very advanced level to provide final quality assurance. It also provided an opportunity for the European Commission to receive the draft documents prior to the workshop discussion.

Attendees:

Tim	Adriaens	Peter	Robertson
Björn	Beckmann	Helen	Roy
Olivier	Blight	Riccardo	Scalera

Etienne	Branquart	Richard	Shaw
Giuseppe	Brundu	Wojciech	Solarz
Thomas	Evans	Alan	Stewart
Marika	Galanidi	Robert	Tanner
Sebastian	Kozic	Elena	Tricarico
Frances	Lucy	Johannes	van Valkenburg
Jodey	Peyton	Hugo	Verreycken
Wolfgang	Rabitsch	Yasmine	Verzelen

The agenda was designed to be flexible but began with a short overview of the project by Helen Roy (Project Lead) followed by a presentation of each risk assessment from the lead author. Guidance on the content required in the presentations for each risk assessment was provided to ensure consistency. Experts were suggested to include a brief introduction to the species followed by a summary of the major sections and the summary assessment. They were also invited to include two final slides to 1. Outline any difficulties with the process and 2. Provide highlights and recommendations from the process.

The presentations were made throughout the first morning. In the afternoon participants gathered together in breakout groups to finalise the risk assessments with the peer-reviewers, on the basis of the presentations and discussions held in the morning. The following day each lead author presented and discussed feedback from the breakout groups activity held the day before, followed by a more general session considering general recommendations and clarifications for the risk assessment template and the management annex.

Workshop discussions on the risk assessments

During the breakout group discussions of the five taxonomic groups at the final workshop of the third year of the contract questions of the assessors were answered and clarified. Further questions, sometimes generic in relation to the template, sometimes specific to the assessed species, were discussed within each group. It was agreed that the template and the guidance have improved over the last two years, however, there are still details that can be further improved. The main issues and points of discussion from the workshop discussions are briefly summarised here.

- The criterion for "Scoring of Likelihoods of Events" (Annex I) is the frequency of events over time (e.g. "likely" = one event in 10 years). While this is comprehensible for the invasion stages of "introduction" and "entry", it is less useful for the "establishment" and especially for the "spread" stage. The Harmonia+ template provides an alternative criterion for assessing the rate of spread and inclusion of this criterion for incorporation into the current template should be considered. Also, the wording of the criterion was questioned and it was suggested to re-phrase the descriptions.
- It was suggested that a question on research needs could be useful. Such a question was included within the previous template versions and removed to reduce the work load of assessors. A possible compromise could be to include a relevant question, but as an "optional" question that need not be answered by assessors.
- It was suggested to develop (or use) a standardized method for reaching the final scores for the conclusions, e.g. in a mathematical way. This suggestion, however,

has been discussed previously and rejected. The instructions in the template state: "*The combination of specific elements (scores) of a risk assessment into a final overall score is difficult. There is no accepted or agreed (or correct) formula or decision protocol for this final step. This risk assessment template uses many different sources of information to deliver assessment scores along the invasion continuum (introduction, entry, establishment, spread, impact), which are not necessarily equal. The conclusion of the risk assessment, however, needs to match the scores of the specific elements in a consistent and sensible way and requires justification of the overall risk*." There is currently no intention to change the template to accommodate this suggestion.
- Qu. 1.3a deals with the "large" numbers of the organisms introduction. It was suggested to replace this by "sufficient" numbers.
- Qu. 1.5a deals with the likelihood of the organisms survival "during transport and storage" beside management practices. It was suggested to add "before and during transport and storage" to cover possible pre-border management activities (e.g. inspections at the source area before exporting goods).
- There was discussion on whether or not factual information should receive a scoring of likelihood. If a species is already present in the risk assessment area, how should the "likelihood of introduction" be scored? The instructions in the template state: "*For organisms which are already present in the risk assessment area, only complete this section [probability of introduction] for current active pathways and, if relevant, potential future pathways*." The questions 1.2-1.7, therefore, refer to pathways and so do the overall scores in 1.8 and 1.9 and the risk summary. One option could be to not assign a score to such cases, but provide an explanation in the comments to the question. Another option would be to use the highest possible score by default. If repeated introductions (in case of active pathways) are possible, it was recommended to give a score and assess the likelihood of introduction into new areas (see also next point).
- A question addressed the scoring for likelihood of introduction for a species that is already present in the risk assessment area, but has no active pathway. The instructions in the template state: "*If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9*."
- There have been difficulties in understanding the different invasion stages "Introduction" and "Entry", specifically for the plant assessments. This is partly due to the fact that the IPPC-template uses different definitions for these invasion stages than the EU-Regulation. It was also highlighted that the EU-Regulation does not separate the "entry" stage. Because different pathways may apply for both stages, it was agreed to keep both sections of the assessment separate.
- There have been difficulties in understanding the definition of "in the wild" with regard to occurrences in gardens (see also COM-FAQ-document stating that: "Plants that are commonly present in gardens will be considered as plants that are established in the environment, because their reproduction and escape is very difficult to prevent (e.g. seeds flying around)." [https://ec.europa.eu/environment/pdf/13_07_2016_QA_en.pdf]. Unfortunately, this interpretation of "established in the wild" does not correspond to standard scientific understandings of the invasion process. It was suggested to replace the term "in the wild" by "in the natural environment" in the template.

- The difference between the scores "Major" and "Massive" for the Magnitude of Enviromental Impacts refers to how "widespread" effects are (Annex II of the risk assessment template). The area under consideration should refer to the area endangered by the organism and not the whole EU-territory.
- It was suggested to change the sequence of the questions and order them not by invasion stage, but answer each question for the invasion stages immediately after each other. There is currently no intention to change the template to accommodate this suggestion.
- It was suggested to cover more than one pathway at the same time if answers are identical. While this seems principally possible, it is hard to imagine that answers to different pathways are completely identical. If it is the case, however, such a procedure seems reasonable.
- It was suggested to delete Qu. 2.5 (and maybe also Qu. 2.6) as this information should be inherently included in Qu. 2.7.
- It was agreed that very unlikely pathways can be mentioned, but need not be assessed.
- It was suggested to move up parts of the information/definitions given in Annexes to the corresponding questions.
- There was some confusion about the separation of the introduction (into the risk assessment area), entry (into the wild), and spread (within the risk assessment area) invasion stages. It was suggested to add more explanations, e.g. introduction and entry are identical for species moved with ballast water.
- It was questioned if the assessment is based on a "worst case" scenario. As this is indeed the case, this general information will be added to the general instructions of the template.
- Also, it was questioned if the assessment of impacts has to be done without considering possible management activities. As this is indeed the case, this general information will be added to the general instructions of the template.
- There was general agreement to simplify and shorten the template (e.g. by reducing the number of questions) as far as possible.

Task 4 Collect evidence on management techniques, implementation costs and cost-effectiveness

Leading experts: Pete Robertson (Newcastle University), Piero Genovesi (IUCN), Dick Shaw (CABI), Marc Kenis (CABI), Riccardo Scalera (IUCN ISSG)

Other contributors: Helen Roy (CEH), Gordon Copp (CEFAS), Wolfgang Rabitsch (EAA), Marianne Kettunen (IEEP), Jørgen Eilenberg (University of Copenhagen), Frances Lucy (Institute of Technology), Franz Essl (EAA), Stefan Schindler (EAA), Tim Adriaens (INBO), Argyro Zenetos (HMRC), Jack Sewell (MBA), Niall Moore (NNSS), Olaf Booy (NNSS) plus other relevant experts.

At the beginning of the project, EC provided a template for the collection of information in relation to management techniques and costs to be used under this task for the compilation of an annex to each of the risk assessments that would inform risk management decisions.

The annexes describe methods for prevention, eradication and management, including a description of the method, evidence for its cost and cost-effectiveness, a measure for the confidence in the available information in each case and a list of bibliographic references.

For the 10 species assessed during 2018/19, there was increased emphasis, compared to previous years of the study, on the inclusion of quantitative information on the costs of management and the maximum areas over which successful management, particularly eradications, have been conducted. These were considered to be particularly useful to inform possible management decisions, however, it was accepted that the availability of published information on these topics, for selected species or their close relatives, was often limited.

During the workshop, a simple analysis of content and whether this was of a quantitative or qualitative nature was presented in relation to the different questions posed in the guidance for the annex. This highlighted a number of topics which could be more consistently covered and this was discussed in relation to the individual species accounts in a subsequent break-out session.

Feedback on the structure of the annexes was also received at this meeting. This included the suggestion to include more sub-headings within the structure of the template to prompt responses on key questions and improve consistency of coverage. A discussion on the definition of methods to include in the annex centred around the role of surveillance and monitoring which have been included in some accounts. The consensus was that these were not to be considered as management options in their own right, and deserved a more lengthy assessment if the information was to be useful which was beyond the role of the management annexes. However, reference should be made in the annex to the role of surveillance and monitoring within the individual management accounts to acknowledge their value and role, for example to support rapid detection. The group also discussed the usefulness of the current structure, given that it does not provide any information on the possible feasibility of management to help inform decision-making.

Conclusions

The risk assessment template developed through the study provided an effective approach to the risk assessment of the ten species prioritised. Of the ten species assessed, four were deemed to constitute a "high" risk (Table 5) all with "medium" confidence. Five species were deemed "moderate" risk with "medium" confidence. *Fallopian baldschuanica* was concluded as "low" risk but only with "low" confidence. The management annexes provided a good basis to be taken into consideration when species will be considered for compliance with the criteria for inclusion on the list of invasive alien species of Union concern.

Table 5. Compilation of the responses (High, Moderate, Low) and confidence (High, Medium, Low) assigned within the conclusion of the risk assessments

Species	*Response*	*Confidence*
Channa argus (northern snakehead)	High	Medium
Ameiurus melas (black bullhead)	Moderate	Medium
Ameiurus nebulosus (brown bullhead)	Moderate	Medium
Wasmannia auropunctata (little fire ant)	High	Medium
Axis axis (chital)	Moderate	Medium
Pycnonotus cafer (red-vented bulbul)	Moderate	Medium
Fallopia baldschuanica (Russian vine)	Low	Low
Phytolacca americana (American pokeweed)	Moderate	Medium
Boccardia proboscidea (polychaete worm)	High	Medium
Schizoporella japonica (orange ripple bryozoan)	High	Medium

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Risk assessment template

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[6]

Name of organism:

Author(s) of the assessment:
including the following elements:
- *name, affiliation, city, country*

Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: *name, affiliation, city, country*
Peer review 2: *name, affiliation, city, country*

Date of completion:

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[6] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

General instructions:

- Completing risk assessments can be time consuming. Risk assessors and peer reviewers should read all questions before completing each assessment to determine where most detail needs to be provided.
- Responses and justifying comments should be concise and directly answer the question being asked.
- The risk assessment shall be based on the most reliable scientific information available, including the most recent results of international research, supported by references to peer reviewed scientific publications. In cases where there are no peer reviewed scientific publications or where the information provided by such publications is insufficient, or to supplement the information collected, the scientific evidence may also include other publications, expert opinions, information collected by Member States' authorities, official notifications and information from databases, including information collected through citizen science. All sources shall be acknowledged and referenced.
- All responses in the risk assessment shall be backed up by primary references. However, as the risk assessment is not a comprehensive review of the biology or ecology of the species but rather needs to assess the relevant information, references to major monographic reviews are acceptable for these points.
- The scoring of the magnitude of impacts (see Annex II) is not identical with the scoring of other risk assessment protocols. For example, the score “Major” in EICAT has a different meaning than “Major” in the present template. Assessors should not copy-paste scores from other protocols without explanations, specifically with regard to the assessment area, and follow the definitions as given in Annex II (see Qu. A.3).
- Questions in the risk assessment should be answered even where there is little information to support a response, with uncertainty in the response clearly discussed. Where there is such a lack of information, the assessor shall state this explicitly.
- Certain questions are not accompanied by specific instructions or explanatory comments as these are sufficiently self-explanatory. Authors should not consider any such questions as less important. In case of doubt or uncertainty, authors may contact for clarification the project co-lead for responsible for the template.
- Each answer provided in the risk assessment shall include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or the fact that the available evidence is conflicting. See Annex III for the documented method.
- The author(s) of the risk assessment and the peer reviewers shall not be affiliated to the same institution.

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a):
Response (6b):

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a):
Response (7b):

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):
Response (8b):

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a):
Response (9b):

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response:

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response:

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: "No information has been found."
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as "corridor" or "unaided)".
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[7] and the provided key to pathways[8].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9

Pathway name:

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	**CONFIDENCE**	low medium

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[7] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[8] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

			high

Response:

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.
Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[9] and the provided key to pathways[10].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.
For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.
In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name:

Qu. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional unintentional	**CONFIDENCE**	low medium high

Response:

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

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[9] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[10] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Response:

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions. Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	very unlikely unlikely moderately likely likely	**CONFIDENCE**	low medium high

	very likely		

Response:

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.
Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	**CONFIDENCE**	low medium high

Response:

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A	**CONFIDENCE**	low

	very unlikely unlikely moderately likely likely very likely		medium high

Response:

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely	**CONFIDENCE**	low medium high

	likely very likely		

Response:

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.
With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic "spread" via release or escape ("jump-dispersal"), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Response:

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Response:

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name:

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	**CONFIDENCE**	low medium high

Response:

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response:

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	**CONFIDENCE**	low medium high

Response:

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	**CONFIDENCE**	low medium high

Response:

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	very slowly slowly moderately rapidly very rapidly	**CONFIDENCE**	low medium high

Response:

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	**CONFIDENCE**	low medium high

Response:

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor	**CONFIDENCE**	low medium

	moderate major massive		high

Comment:

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found". Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor	**CONFIDENCE**	low medium

	moderate major massive		high

Comments:

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

RISK SUMMARIES

	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Introduction*	very unlikely unlikely moderately likely likely very likely	low medium high	Provide a comprehensive short summary of your response to Questions 1.8-1.9.
Summarise Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	Provide a comprehensive short summary of your response to Questions 2.8-2.9.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	Provide a comprehensive short summary of your response to Questions 3.13-3.14.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	Provide a comprehensive short summary of your response to Questions 4.11-4.12.
Summarise Impact*	minimal minor moderate major massive	low medium high	Provide a comprehensive short summary of your response to Question 5.19-5.20.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	The combination of specific elements (scores) of a risk assessment into a final overall score is difficult. There is no accepted or agreed (or correct) formula or decision protocol for this final step. This risk assessment template uses many different sources of information to deliver assessment scores along the invasion continuum (introduction, entry, establishment, spread, impact), which are not necessarily equal. The conclusion of the risk assessment, however, needs to match the scores of the specific elements in a consistent and sensible way and requires justification of the overall risk.

*in current climate conditions and in foreseeable future climate conditions

REFERENCES

Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria					
Belgium					
Bulgaria					
Croatia					
Cyprus					
Czech Republic					
Denmark					
Estonia					
Finland					
France					
Germany					
Greece					
Hungary					
Ireland					
Italy					
Latvia					
Lithuania					
Luxembourg					
Malta					
Netherlands					
Poland					
Portugal					
Romania					
Slovakia					
Slovenia					
Spain					
Sweden					
United Kingdom					

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment	Possible establishment	Invasive (currently)

			(under current climate)	(under foreseeable climate)	
Alpine					
Atlantic					
Black Sea					
Boreal					
Continental					
Mediterranean					
Pannonian					
Steppic					

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea					
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast					
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea					
Aegean-Levantine Sea					
Ionian Sea and the Central Mediterranean Sea					
Western Mediterranean Sea					

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[11]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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[11] Not to be confused with "no impact".

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes;

			Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)*
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[12]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure)

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[12] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

		anthropogenic origin	*Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*
		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions**	**Physical and experiential** interactions	Characteristics of living systems that that enable activities promoting health,

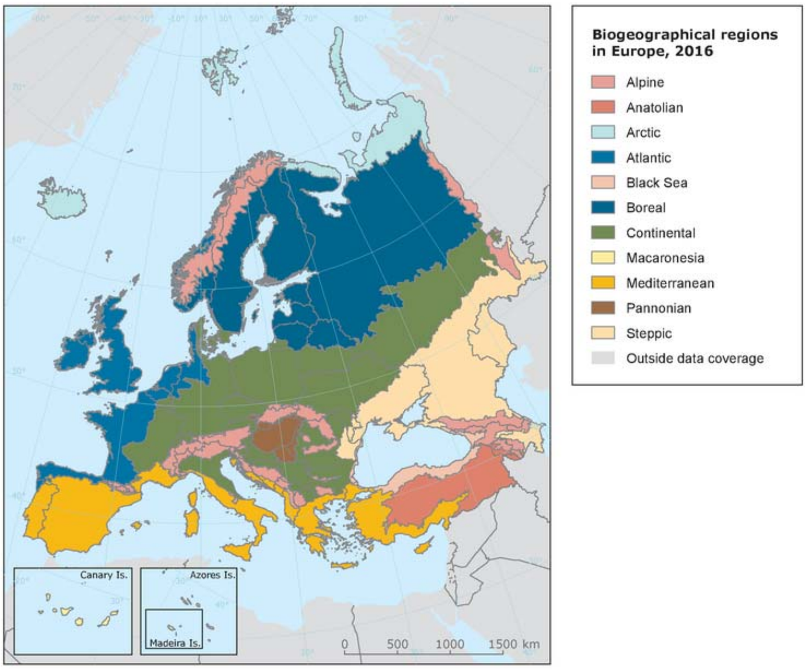
	with living systems that depend on presence in the environmental setting	with natural environment	recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

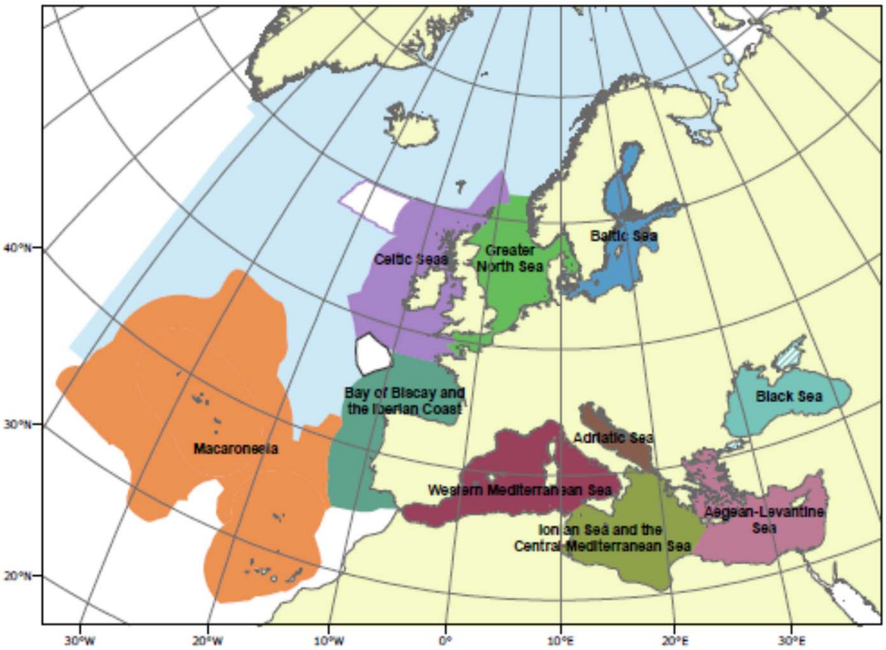
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: *Channa argus* (Cantor, 1842)

Authors of the assessment:

- *Luke Aislabie, Cefas, Lowestoft, U.K.*
- *Hugo Verreycken, Research Institute for Nature and Forest (INBO), Brussels, Belgium*
- *Gordon H. Copp, Cefas, Lowestoft, U.K*

Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: *John S. Odenkirk, Virginia Department of Game and Inland Fisheries, Fredericksburg, VA USA*

Peer review 2: *Jeffrey Hill, University of Florida, Ruskin, Florida, USA*

Date of completion: 23 October 2019

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: *Channa argus* (Cantor, 1842) is clearly a single taxonomic entity and it can be adequately distinguished from other species of the same genus (Courtenay & Williams, 2004).

Kingdom: Animalia –animals

Subkingdom: Bilateria

Infrakingdom: Deuterostomia

Phylum: Chordata –chordates

Subphylum: Vertebrata –vertebrates

Infraphylum: Gnathostomata

Superclass: Osteichthyes – bony fishes,

Class: Actinopterygii – ray-finned fishes

Subclass: Neopterygii – neopterygians Infraclass: Teleostei

Superorder: Acanthopterygii

Order: Perciformes – perch-like fishes

Suborder: Channoidei

Family: Channidae – snakeheads

Genus: *Channa* Scopoli, 1777 – Asian snakeheads

Species: *Channa argus* (Cantor, 1842)

The preferred common name in English is northern snakehead. Other English names are Amur snakehead, eastern snakehead and snakehead (Froese & Pauly, 2019).

The only valid scientific name is *Channa argus* Cantor, 1842 (Froese & Pauly, 2019). The previously described subspecies *Channa argus argus* Cantor, 1842 and *Channa argus warpachowskii* (Berg, 1909) are not considered valid anymore nor are the other synonyms and combinations.

Non-valid senior and junior synonyms are *Channa argus kimurai* Shih, 1936, *Ophicephalus argus* Cantor, 1842, *Ophicephalus nigricans* Cuvier, 1831, *Ophicephalus pekinensis* Basilewsky, 1855, *Ophiocephalus argus* Cantor, 1842, *Ophiocephalus argus warpachowskii* Berg, 1909.

In ornamental aquatic trade the common name "platinum snakehead" is used for *Channa argus kimurai*, sometimes also referred to as *C. argus* "platinum"[2] or *C. argus var* 'Kimnra'[3].

This risk assessment considers the species *C. argus* with all its non-valid senior and junior synonyms.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

Other snakeheads (*Channa* spp,) are superficially similar to *C. argus.* These are all alien to and not established in the risk assessment area. Some species of genus *Channa* are traded and kept in aquaria within the risk assessment area. There are no similar native species in the risk assessment area.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

At least four RAs are available for North America and might be of interest and/or partly valid for the risk assessment area as conditions are similar in parts of N. America and the risk assessment area.

Courtenay and Williams (2004) included the biological and risk information used to list the family Channidae (snakeheads) as injurious in the United States

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[2] See https://www.ruinemans.com/en/product/09295-channa-argus-platinum-l

[3] See e.g. https://animalscene.ph/2018/02/26/searching-for-the-true-identity-of-the-platinum-snakehead/ and https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2518323

(https://pubs.er.usgs.gov/publication/cir1251). They assessed the probability of establishment and the consequences of establishment and the organism risk potential and rated them all high with a moderate to high certainty. They conclude that Channidae are organisms of major concern to the USA and constitute an unacceptable risk that justifies mitigation.

Cudmore and Mandrak (2006) assessed northern snakehead for Canada (http://biblio.uqar.ca/archives/30163321.pdf) . They also assessed the probability of establishment and the consequences of establishment and rated the organisms risk potential to be high with a reasonable certainty.

In a trinational risk assessment for North America (CEC 2009; http://www3.cec.org/islandora/en/item/2379-trinational-risk-assessment-guidelines-aquatic-alien-invasive-species-en.pdf), the assessors again rate northern snakehead as a high risk species with high probability of establishment (uncertainty: very certain) and high consequences of establishment (uncertainty: reasonably certain).

Northern snakehead was screened in an Ecological Risk Screening Summary for the United States (USFWS, 2017) (https://www.fws.gov/fisheries/ANS/erss/highrisk/Channa-argus-ERSS-FINAL-Sept-2017.pdf) and this resulted in categorizing it as a high overall risk species (with high climate match and a high history of invasiveness, with medium certainty).

In relation to the risk assessment area, *C. argus* has been ranked, using the Fish Invasiveness Screening Kit (FISK) decision-support tool (Copp et al., 2009), as posing a high risk of being invasive in the following risk assessment areas: England & Wales, the Iberian Peninsula and southern Finland (Vilizzi et al., 2019). Vilizzi et al. (2019) also reported a medium risk for Australia and Japan and a high risk for Florida (USA).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: The native range of the northern snakehead is in Asia in the Amur southward to Xi Jiang and Hainan Island, China (Bogutskaya et al. 2008). FishBase (Froese & Pauly, 2019) mentions China, (South) Korea and Russia as native countries.

Fig. 1: Distribution of *C. argus* in the Eastern Hemisphere (Native and introduced range) Source: Courtenay & Williams (2004).

Courtenay and Williams (2004) made a literature review and summarise the native range of *Channa argus* (Cantor, 1842) as: Middle and lower Heilong (Amur), Songhua (Sungari), Manchuria, Tunguska (at Khabarovsk, Russia) and Ussuri; Lake Khanka; throughout Korea except its north-eastern region; rivers of China southward and south-westward to upper tributaries of the Chang Jiang (Yangtze) River basin in north-eastern Yunnan Province. Reported from Guangdong Province, China, likely an introduction there. Widely distributed in Chinese reservoirs.

It is as good as impossible for the northern snakehead to naturally spread into the risk assessment area from its native range.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: The global non-native range of northern snakehead is not very clear and can differ between contacted sources. FishBase (Froese & Pauly, 2019) state Japan, Turkmenistan, Uzbekistan and the USA as non-native countries. Bogutskaya and Naseka (2002) mention *C. argus* to have been transplanted within Russia to ranges where it is non-native but establishment there failed.

Northern snakehead was brought from Korea and intentionally released by culturists in Japan in the early 1900s (Okada, 1960). In Kazakhstan, Turkmenistan, and Uzbekistan release in ponds, rivers, and reservoirs in the early 1960s may have been accidental via transport in contaminated shipments of Asian carps (Courtenay & Williams, 2004), northern snakehead subsequently became established in these waters.

The introduction into the USA is best documented, the first records of northern snakehead date from the late 1990s. The species is established in the Mid Atlantic region (Virginia tributaries of the Potomac River) and in Arkansas (Benson, 2019; Froese & Pauly, 2019; Odenkirk & Isel, 2016).

Within the risk assessment area, *C. argus* was introduced in the Czech Republic (former Czechoslovakia) in 1956 but is probably not established there (Lusk et al., 2010) while Musil et al. (2010) even state that *C. argus* is now extinct in the Czech Republic (see Q. A8).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, including the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to: www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to: http://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a): Pannonian, see answer to Q.8(a)

Response (6b): The species is currently not established in any part of the risk assessment area.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9–2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

- Response (7a): Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic
- Response (7b): Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Northern snakehead has a wide temperature range (0°C - 30°C, optimum 10 – 27°C) (Courtenay & Williams, 2004) and it exhibits a broad tolerance to a wide range of environmental conditions and is extremely hardy (Cudmore & Mandrak, 2006) so it can probably establish in most of the EU biogeographic regions. No climate change scenarios are available for northern snakehead for the risk assessment area but because of its broad environmental tolerance it can be assumed that it would still be able to establish under climate change conditions in the same biogeographic regions as under the current conditions.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): Northern snakehead was introduced in the Czech Republic (former Czechoslovakia) in 1956 as part of an experimental stocking programme but establishment failed (Lusk et al., 2010). Musil et al. (2010) even state that *C. argus* is now extinct in the Czech Republic.

Response (8b): None

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9–2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, France, Germany, Greece, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, United Kingdom.

Response (9b): Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Northern snakehead has a wide temperature range (0°C - 30°C, optimum 10 – 27°C) (Courtenay & Williams, 2004) and it exhibits a broad tolerance to a wide range of environmental conditions and is extremely hardy (Cudmore & Mandrak, 2006) so it can probably establish in most of the risk assessment area countries. No climate change scenarios are available for northern snakehead for the risk assessment area but because of its broad environmental tolerance it can be assumed that it would be able to establish in all countries under climate change conditions.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: Northern snakehead is considered invasive by many authors (e.g. Bressman et al. (2019) and Love & Genovese (2019) for the USA) but not by others (e.g. Nakai (2019) for Japan). Vilizzi et al. (2019) assessed northern snakehead to be of medium risk of becoming invasive for Australia and Japan and of high risk for Florida (USA).

The species' high fertility and tolerance of a wide range of conditions, as well as the reduced number of natural enemies in its introduced range, make it highly likely to be a formidable invasive if it was to become established (Global Invasive Species Database, 2020).

The family Channidae is included in Species Listed as Injurious Wildlife under the Lacey Act (18 U.S.C. 42a). Species listed as injurious may not be imported or transported between the continental United States, the District of Columbia, the Commonwealth of Puerto Rico, or any territory or possession of the U.S. by any means without a permit issued by the Service (Hill et al., 2018). Permits may be granted for the importation or transportation of live specimens of injurious wildlife and their offspring or eggs for bona fide scientific, medical, educational, or zoological purposes.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: The species is not yet known to be present in the risk assessment area.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response: The species is not yet known to be present in the risk assessment area.

Within the risk assessment area, although not established, the species is regulated as potentially invasive by some Member States e.g. import and sale is banned in England and Wales[4]. Furthermore, the entire *Channa* genus is included in the Spanish[5] and Portuguese[6] national catalogues of invasive alien species.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response: Some snakehead species are used in the aquarium fish trade, especially small species and brightly coloured juveniles of several large snakeheads, e.g. giant snakehead *Channa micropeltes* (Zięba et al., 2010). However *C. argus* is not very popular with aquarists because they attain a large size and are difficult to feed (Cudmore & Mandrak, 2006). Snakeheads are moderately popular with hobbyists in Japan and Europe. There are no economic data available for northern snakehead in the aquarium trade, but the trade value is probably very low.

Channa species are important for aquaculture, for instance Halwart et al. (2009) report a production of 11525 tonnes of *C. micropeltis* in cages (all countries except China). They also mention that *Channa* species are mainly cultivated in Cambodia and Vietnam. On FishBase (Froese & Pauly, 2019) (https://www.fishbase.de/report/FAO/FAOAquacultureList.php?scientific=Channa+argus) the FAO statistics for the aquaculture production of *C. argus* in China and Korea can be found. Mean yearly production in China (2003 – 2007) is 230,000 tonnes and in the Republic of Korea (1976 – 2007) about 300 tonnes. Zhuo et al. (2012) report that northern snakehead is renowned as a food fish in China due to its good taste, high protein content and few intramuscular bones. It is also regarded as a good tonic food fish used in traditional medicine for wound-healing.

Several species are marketed in Canada and have been sold in the U.S.A., even in states where possession of live snakeheads has been illegal for decades (Courtenay & Williams, 2004). Hobbyists and importers can purchase snakeheads through a variety of sites on the Internet, also in Europe. Because of their highly predacious nature, however, snakeheads have not had a large following of interested hobbyists in the U.S.A. (Courtenay & Williams, 2004). The trade is generally illegal in most

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[4] The Prohibition of Keeping or Release of Live Fish Order 2003 http://www.legislation.gov.uk/uksi/2003/25/article/2/made

[5] Real Decreto 630/2013, de 2 de agosto, por el que se regula el Catálogo español de especies exóticas invasoras.

[6] Decreto-Lei n.º 92/2019 de 10 de julho

of the USA (some states do not prohibit and there are a few remaining stocks perhaps) and the trade for aquarium hobbyists is tiny to non-existent now (J. Hill, pers. comm.).

Prior to Federal regulations restricting importation of the species, *C. argus* was the most widely available snakehead sold as a live-food fish in the U.S. accounting for the largest volume and greatest weight of live snakeheads imported into the U.S. until 2001 (Courtenay & Williams, 2004).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: "No information has been found."
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as "corridor" or "unaided)".
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[7] and the provided key to pathways[8].
- For organisms that are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Q. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context, a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2–1.9

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[7] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[8] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Known pathways for introduction of *C. argus* are:

ESCAPE FROM CONFINEMENT - Pet/aquarium/terrarium species

ESCAPE FROM CONFINEMENT – Live food and live bait

RELEASE IN NATURE - Fishery in the wild (including game fishing)

RELEASE IN NATURE - Other intentional release (ceremonial release as a prayer species)

In other parts of the world, *C. argus* were intentionally introduced for aquaculture and aquarium trade and released for angling purposes and as prayer species (Cudmore & Mandrak, 2006). For the risk assessment area, however, there are no current active pathways of introduction of *C. argus* described.

Below we will discuss only the pathway assumed to be or become the most important (aquarium trade) as the other pathways are estimated to be non-existing in the risk assessment area and not to become important in the near future. Currently, there seems to be no aquaculture or fishing interest for *C. argus* in Europe and very few *C. argus* (if any) would be available in the risk assessment area for prayer animal release. Also, in the risk assessment area natural range extension will not be possible since there are no confirmed established populations.

Pathway name: ESCAPE FROM CONFINEMENT - Pet/aquarium/terrarium species

Q. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**intentional** unintentional	**CONFIDENCE**	low medium **high**

Response: Aquarists in Japan, Europe, including the U.K. (e.g. *C. micropeltes*; Zięba et al., 2010), and to a lesser extent North America, have kept small, colourful snakehead species as pet fish (Courtenay & Williams, 2004). However, *C. argus* is not favoured for the aquarium or water garden trade as they are not very colourful and rapidly attain very large sizes (Courtenay & Williams, 2004; Orrell & Weigt, 2005). The same can be said of *C. micropeltes* in the U.K., where a dead specimen was found on a river bank, presumably released live to the water by its owner when the pet aquarium fish became too large (Zięba et al., 2010).

Q. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: There is online trade outside (*C. argus*, http://aquariumfishexporter.com/products/tropical-fish/snakehead/) and inside the risk assessment area (www.ruinemans.com/en-GB/7765/Channa-argus-platinum-l.html. However, *C. argus* is not favoured for the aquarium or water garden trade as they are not very colourful and rapidly attain very large sizes (Courtenay & Williams, 2004; Orrell & Weigt, 2005; Zięba et al., 2010); other *Channa* species are clearly introduced more frequently for the aquarium trade. No quantitative data on live shipments of *C. argus* were found for the risk assessment area.

Q. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Survival is estimated to be high as the transport of live fish is normally well organised and the conditions for aquarium fish are normally very good. But even in bad conditions, *C. argus* would probably survive as *C. argus* exhibits a broad tolerance to a wide range of environmental conditions and is extremely hardy (Cudmore & Mandrak, 2006). According to Courtenay and Williams (2004) in a consignment of *C. argus*, the fish were even alive after being shipped from China without water to Canada. The potential for *C. argus* to survive in transit while being shipped overseas is high. Many snakehead species are obligate air breathers, others are facultative air breathers. Therefore, some snakehead species are capable of surviving hypoxic conditions and can even survive out of water for considerable periods of time as long as they remain moist (Mendoza et al., 2009). However, reproduction or an increase in numbers will not occur during transport.

Q. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: *Channa argus* is extremely hardy and exhibits considerable tolerance to a wide range of environmental conditions (Cudmore & Mandrak, 2006), so it is likely that they would survive existing management practices during transport and storage along the pathway.

Q. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: Other species of *Channa* are present in the aquarium trade, e.g. *C. micropeltes,* and some specimens of *C. argus* could be imported as a contaminant of the intended consignments, or if the species in the consignments are mislabeled or erroneously identified as a *Channa* species other than *C. argus*. This latter case occurred with the Asian weatherfish (*Misgurnus mizolepis*), which was imported to the U.K. under the name *Misgurnus anguillicaudatus* (Zięba et al., 2010). However, *C. argus* is a species from temperate waters, and the *Channa* species sold in the aquarium trade, e.g. *C. micropeltes*, are normally tropical species.

Q. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: *Channa argus* is of low importance in the aquarium fish trade, with the species not being favoured for aquaria or water gardens due to the fact that they are not very colourful, except as small juveniles, and they rapidly attain very large sizes (Courtenay & Williams, 2004; Orrell & Weigt, 2005). The number of imported specimens of this species through this pathway would thus be low.

End of pathway assessment, repeat Q. 1.3 to 1.7 as necessary using separate identifier.

Pathway name: ESCAPE FROM CONFINEMENT – Live food and live bait

Q. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**intentional**	**CONFIDENCE**	**high**

Response: The importation of *C. argus* to the U.S.A. was originally as live fish for the Oriental retail and restaurant trade (Courtenay & Williams, 2004), and in view of the existence of an Asian market in virtually all EU countries, this species could be of interest amongst importers for the wholesale or retail live fish trade.

Q. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1–2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: For the trade in an imported live fish to be commercially viable, the consignments would need to be relatively large.

Q. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: The purpose of a live fish import is to have live fish to sell, and the species is known to be very tolerant of low oxygen levels and other stressors, so the likelihood of survival is high but reproduction and an increase in numbers would not take place during transport.

Q. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: As mentioned in the response to Q 1.4b, the purpose of a live fish import is to have live fish to sell, so management practices during transport and storage can be assumed to be designed to ensure the fish arrive in a living state.

Q. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: The accidental importation of live fishes as “contaminants” of a consignment of another fish species is possible, but this would seem unlikely.

Q. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: Although the likelihood of survival, of high numbers etc. range from moderately likely to very likely, these depend upon the species actually being imported to the risk assessment area in a live form for commercial or other sale. At present, there is no known importation of *C. argus* in live form to the risk assessment area, and therefore, overall, the species’ introduction into the risk assessment area via this pathway is unlikely.

Q. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: Although the likelihood of survival, of high numbers etc. range from moderately likely to very likely, these depend upon the species actually being imported to the EU in a live form for commercial or other sale. At present, there is no known importation of *C. argus* in live form to the risk assessment area, and therefore, overall, the species' introduction into the risk assessment area via this pathway is unlikely.

Q. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30–50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9–2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: Because the aquarium/pet fish trade is not normally influenced by climate, and there does not appear to be any particular commercial pressure for the importation of live *C. argus* to the risk assessment area for consumption, it is unlikely that there would be any difference in the introduction of this species in the future.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[9] and the provided key to pathways[10].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Q. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2–2.8

Pathway name: ESCAPE FROM CONFINEMENT - Pet/aquarium/terrarium species

Q. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	**intentional** unintentional	**CONFIDENCE**	low medium **high**

Response: Some snakeheads living in natural waters of the U.S. may have been released by aquarium hobbyists (USGS, 2004). As such, if the trade of *C. argus* for aquarium purposes into the risk assessment area were to become important, which is unlikely as *C. argus* is not favoured for the aquarium or water garden trade as they are not very colourful and rapidly attain very large sizes (Courtenay & Williams, 2004; Orrell & Weigt, 2005), then deliberate release from aquaria would be likely. The likelihood of release is further increased due to the highly-predacious nature and the significant costs associated with feeding and housing of this species (Cudmore & Mandrak, 2006).

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[9] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[10] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Long-living, large-bodied species have a higher chance of being released into the environment from aquaria and garden ponds (Magalhães et al., 2017).

Q. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: Because snakeheads, in particular *C. argus*, represent only a very minor component of aquarium fish trade, the illegal release or dumping of this species in the environment will never encompass large numbers of individuals.

Q. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Illegal disposal of fishes is probably not going to be reported.

Q. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Disposal of unwanted fish (e.g. too big for aquarium) is very likely to happen any time of year. Since *C. argus* is a warm to cold water species (Froese & Pauly, 2019) it could thrive the whole year round in most of the risk assessment area. This is supported by the assessment of Cudmore and

Mandrak (2006) of the possible distribution of *C. argus* in Canada using models based a.o. on temperature and suggest that the distribution of *C. argus* could be widespread in Canada even up to about 60°N.

Q. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Disposed fish are very likely to end up in waters in close proximity of the aquarium. The aquarium trade has been identified as an important pathway of aquatic invasive species (Maceda-Veiga et al., 2013).

Q. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Although the illegal release or dumping of this species in the environment will never encompass large numbers of individuals, it is likely that *C. argus* will enter into the environment via this pathway. This species is difficult to hold in aquaria because of their highly predacious nature and the significant costs associated with feeding and housing this species (Courtenay & Williams 2004). Snakeheads are therefore likely to end up in the environment after, or as a consequence of, illegal release (Copp et al., 2005b).

End of pathway assessment, repeat Q. 2.2 to 2.7. as necessary using separate identifier.

Pathway name: ESCAPE FROM CONFINEMENT – Live food and live bait

Q. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	**intentional**	**CONFIDENCE**	**high**

Response: Although escape from live fish holdings remain a distant possibility, the fact that such fish are normally held in quarantine-type locations, is not considered here. However, there have been cases of releases of live animals, imported live for consumption, such as the American lobster *Homarus americanus*, which were released at various locations around Great Britain but with large numbers at two locations along the southern coast of England (Stebbing et al., 2012). There are various reasons for the live release of animals, including animal rights and religious beliefs (Copp et al., 2005b; Stebbing et al., 2012).

Q. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	moderately likely	**CONFIDENCE**	**medium**

Response: The intentional release of live animals that have been purchased for that purpose is most likely to be as part of a religious ceremony or to make an animal-rights political statement (Crossman & Cudmore, 1999; Copp et al., 2005b). In either case, it can be assumed that the release of a large number of animals would make a bigger impact (religious or political) than a single individual or a few specimens. So, it is moderately likely that a relatively large number of fish would be released into the environment.

Q. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Illegal disposal of fishes is probably not going to be reported, so the sole detection would be by those persons releasing the fish.

Q. 2.5b. How likely is the organism to enter into the environment during the months of the

year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: It would seem counter-productive for the release of live animals (as a religious or political statement) to take place at a time of year when the fish are likely to die due to thermal shock.

Q. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Again, it would seem counter-productive for the release of live animals (as a religious or political statement) to be into a habitat that is not suitable for that species. That said, if the persons undertaking the release are not familiar with *C. argus* and release the fish into a marine environment, then the fish would die. Note that there is conflicting information as regards to the salinity tolerance of *C. argus*. Courtenay and Williams (2004) reported it to be 1–10 ppm, whereas Fuller et al. (2019) have reported the upper limits to be 15–18 ppt. Bunch et al. (2019) state that the upper lethal limit is 18 ppt, they can disperse through 5-18 ppt, but probably persist mostly below 10 ppt.

Q. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: Assuming that the species is in fact introduced to the risk assessment area in live form, then intentional releases to the environment, whether intentional or accidental, are moderately likely, based on past events with *C. argus* (Courtenay & Williams 2004) and other non-native aquatic animals (Copp et al., 2005b; Stebbing et al., 2012).

Q. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: If *C. argus* is actually imported to the risk assessment area in live form, then it is moderately likely that it will be released, or will escape, into the environment at some point.

Q. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: If *C. argus* is actually imported to the risk assessment area in live form, then it is moderately likely that climate will not affect the likelihood of it being released, or to escape, into the environment.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Q. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	**very likely**	**CONFIDENCE**	**medium**

Response: *Channa argus* has a broad range of environmental tolerances and is extremely resilient; it inhabits fresh waters within a temperature range of 0 to 30°C (Courtenay & Williams, 2004), preferring stagnant shallow ponds or swamps with mud substratum and vegetation; they can also be found in slow muddy streams and in canals, reservoirs, lakes, and rivers (Cudmore & Mandrak, 2006; Dukravets & Machulin, 1978). As an obligate airbreather it can survive out of water for up to four days by breathing oxygen; cold temperatures reduce metabolism rates and oxygen demand, allowing them to survive under ice (Global Invasive Species Database (2020). In the U.S.A., *C. argus* has reported in the New England states of Massachusetts (Courtenay & Williams, 2004) and Maine (Fish and Wildlife Service, 2002), as well as California, Florida, North Carolina, Rhode Island, and Wisconsin (CABI, 2012), with established populations reported for the state of Maryland (Landis et al., 2011) and in Arkansas (Rypel, 2014). The species has also been found in British Columbia, Canada (Scott et al., 2013). A prediction map for the U.S.A. (Poulos et al., 2012) indicates that suitable habitat for *C. argus* exists from Mexico to Hudson Bay, New York State (Herborg et al., 2007). Therefore, *C. argus* could spread to warmer parts of the southeastern United States and Florida, and because most (if not all) of the climate types in that geographical span match those of the risk assessment area (Peel et al., 2007), most of the risk assessment area is at risk of *C. argus* establishment. Female *C. argus* in the Potomac River began spawning at the end of April and continued through August, with a peak spawning at the beginning of June, when mean temperatures were 26ºC. *Channa argus* in the Potomac River demonstrated plasticity in timing of reproduction, which may be bi-modal, and rapid larval growth rates (Landis et al., 2011), attributes that are likely to contribute to the species establishment success in novel environments.

Q. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	**widespread**	**CONFIDENCE**	**high**

Response: There is an abundance of suitable habitats for *C. argus* throughout the risk assessment area, where lentic habitats and regulated rivers are very common in central and southern regions.

Q. 3.3. If the organism requires another species for critical stages in its life cycle, then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	**N/A**	**CONFIDENCE**	**high**

Response: No evidence of a dependency on any other species.

Q. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: *Channa argus* is known to be 'highly predatory' (Courteney & Williams, 2004), with fish representing up to 33% of their diet (Okada, 1960; R/C Modeler Corporation, 2010), spanning 17 species of prey fish, including loaches, breams, common carp *Cyprinus carpio*, and Eurasian perch *Perca fluviatilis* (Dukravets & Machulin, 1978). Other than fishes, the species' diet includes crayfishes, dragonfly larvae, beetles, and frogs, all of which are present throughout the risk assessment area. Combined with the species' ability to make short overland movements (Scott et al., 2013), this suggests that the species is likely either to devour or to out-compete native species, especially after entering an enclosed water body – a similar phenomenon has been reported in England for small ponds following the release of northern pike *Esox lucius*, a native piscivorous fish species (Copp et al., 2005b).

In the U.S.A., a study to quantify *C. argus* diet relative to those of non-native largemouth bass *Micropterus salmoides*, and native American eel *Anguilla rostrata* and yellow perch *Perca flavescens* in tidal freshwaters of Virginia and Maryland (Saylor et al., 2012), found that >97% of *C. argus* gut contents were fishes, with fundulid and centrarchid species consumed most frequently. Dietary overlap was biologically significant only between *C. argus* and non-native *M. salmoides*. Aquatic invertebrates were >10 · more common in native predator diets, reducing dietary overlap with *C. argus*.

Q. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	moderately likely	**CONFIDENCE**	medium

Response: Unlike U.S. highly predatory native fishes, snakeheads are very protective of their young, thus enhancing survival beyond early life history stages and suggesting the possibility of eventual dominance in suitable waters (Courtenay & Williams, 2004). Native piscivorous fishes in the risk assessment area that could prey on *C. argus* include northern pike *Esox lucius* and Eurasian perch

Perca fluviatilis, with piscivorous birds such as cormorants and herons, but there is no evidence to suggest that these species would hinder *C. argus* establishment.

We are not aware of any studies that examined whether pathogens and parasites already present in the non-native range could prevent or minimise snakehead establishment.

However, snakehead mortality in intensive culture, such as *C. argus*, but particularly chevron snakehead *Channa striata* and spotted snakehead *Channa punctata*, has been known to occur from epizootic ulcerative syndrome (EUS), a disease which involves several pathogens. This disease is the only one that was highlighted by Courtenay & Williams (2004). There are no known studies of whether EUS is observed in wild populations or whether it could become a limiting factor for the establishment of the species in the risk assessment area.

Q. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: As with virtually all fishes, it would be virtually impossible to eradicate *C. argus* once it was established in a water course. However, in small, closed waters (e.g. small lakes or ponds), eradication may be possible by chemical means (e.g. rotenone) or drain down of the water body (Britton et al., 2008, 2010), especially if undertaken immediately prior to spawning (Jiao et al., 2009).

Q. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: Monitoring and detection approaches currently being used at the national level are unlikely to detect the species, if introduced to the risk assessment area and released to the environment, and therefore establishment of *C. argus* is likely to establish before detection and management measures can be arranged to extirpate the species. Thus, existing management practices are moderately likely to facilitate establishment.

Q. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: If the species is released to a large water course, then eradication is very unlikely to be successful. However, in small water bodies, use of rotenone is likely to extirpate the species due to its high sensitivity to that chemical piscicide (Lazur et al., 2006). Other stressors, such as low oxygen concentration are unlikely to have any effect due to the species ability to survive extended periods of ice cover (Courtenay & Williams, 2004).

Q. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: A mature *C. argus* female can carry as many as 115 000 eggs (Dukravets & Machulin, 1978), with spawning taking place in somewhat dense aquatic vegetation where they feed and reproduce (Courtenay & Williams, 2004). Depending on water temperature, eggs can hatch in about 24–48 hours. When the young-of-the-year *C. argus* hatch, they remain clustered near the nest for 3–4 weeks, protected by their parents, until their fins develop. At that time, early juveniles begin swimming by diving down into the centre of the nest, then rising back to the surface. Early juveniles remain in the nest for 3–4 weeks, schooling, and being guarded by one or both parents. All species of snakeheads guard their eggs and young, a behaviour that is rare in our native fishes. Juvenile *C. argus* cluster at the surface of their "nest," a column of water cleared from vegetation in 0.5–0.75 m of water. *Channa argus* parents will aggressively guard their nest for 3–4 weeks while the young-of-the-year fish develop their fins, learn to school, and are ready to fend for themselves (FWS, 2004)

Channa argus reaches sexual maturity at 2–3 years of age, i.e. 30 to 35 cm total length (TL). Females are iteroparous (repeated reproductive events) and are capable of spawning one to five times per year (Courtenay & Williams, 2004). Fecundity is variable and ranges from 1300–15 000 eggs (mean number of eggs = 7300) per spawning event. Fecundity of individuals ranges from 21 000 to 51 000 per event, often exceeding 100 000 eggs produced annually (Frank, 1970). This high fecundity facilitates the species' rapid establishment in novel environments. *Channa argus* is a long-lived fish species, with one specimen recorded as attaining eight years of age and a length of 760 mm TL which indicates multiple spawning occasions (Courtenay & Williams, 2004; Froese & Pauly, 2019).

Q. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Cudmore and Mandrak (2006) report that *C. argus* has a greater temperature tolerance than most other fish species, is highly adaptable to a wide range of environments (evidenced by its establishment in waters all over Asia), reproduces at a high rate (one female can produce 100,000 eggs a year), and feeds on a wide variety of fish of all sizes, shrimps, prawns, crabs, and insect larvae (Hilton, 2002).

Q. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: This has already happened in the U.S.A., where in April 2004, several fish were found from the Potomac River in Maryland and Virginia (USGS, 2004). It has been determined that these populations were the result of several independent introductions and that the populations are reproducing naturally (Odenkirk & Owens, 2005; Orrell & Weigt, 2005). Wegleitner et al. (2016) suggest that two genetic populations of *C. argus* exist in the eastern United States, possibly as a result of two unique introductions from a source population in its native range or from some other undiscovered and unsampled population not included in their dataset. Successful establishment by two separate founder releases in the U.S.A. does suggest that initial establishment is possible based on low genetic diversity. However, these results fare insufficient to answer with certainty that low genetic diversity would not impede establishment over the longer term, given that true establishment refers to continued persistence of the new population.

Q. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: The species ability to breath air, and its high tolerance to many other environmental conditions indicates that even if establishment is unsuccessful, then the extant *C. argus* will persist as casuals until their death (Courtenay & Williams 2004).

Q. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Comparison of the habitats and climates of the risk assessment area and where *C. argus* has established outside its native range (Peel et al., 2007), e.g. the U.S. (Courtenay & Williams 2004), it is very likely the species would be able to establish itself if imported live to the risk assessment area and released to the environment under current climate conditions. Northern snakehead is the only temperate snakehead species, and tolerance for a wide range of environmental conditions could allow this species to survive in most regions of North America, from northern Florida to Hudson Bay and Alaska and likely in other temperate regions such as western Europe (Lapointe et al., 2013°.

Q. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.
With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30–50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9–2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: As stated here above, comparison of the habitats and climates of the risk assessment area and where *C. argus* has established outside its native range (Peel et al., 2007), e.g. the U.S. (Courtenay & Williams 2004), indicates that it is very likely the species would be able to establish itself if imported live to the risk assessment area and released to the environment under future climate conditions.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic "spread" via release or escape ("jump-dispersal"), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Q. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	**major**	**CONFIDENCE**	**medium**

Response: *Channa argus* is capable of short overland migration (Cudmore & Mandrak, 2006; Scott et al., 2013), although it has not been observed in introduced populations in the United States (J. Hill, pers. comm.), and downstream migrations have also been reported in non-native populations (Courtenay & Williams 2004). Lapointe et al. (2013) demonstrated that in the Potomac River in the invaded range in the USA *C. argus* remain in restricted home ranges throughout the year, but that a considerable portion of the population can disperse over considerable distances to establish a new home range. If introduced to the risk assessment area and escaped or was released to open waters, then *C. argus* is expected to similarly be able to spread via natural means, both from still waters to water courses and within water courses as also in Europe most rivers are interconnected by man-made waterways.

Q. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the

environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	**minor**	**CONFIDENCE**	**medium**

Response: Although anglers were considered responsible for the spread of *C. argus* in several locations within their native and introduced ranges (Courtenay & Williams, 2004), there seems to be very little interest in snakehead fishery in the risk assessment area, therefore the risk of spread through this pathway seems to be minimal in the risk assessment area.

The release of captive larger-sized *Channa* species to open waters has already been demonstrated in one EU country, i.e. *C. micropeltes* in the U.K. (Zięba et al., 2010), so disposal of unwanted fish from aquaria is probably of greater likelihood as a risk of spread. However, *C. argus* seems to be currently of little interest for aquaria in the risk assessment area.

Overall, angler and aquarist releases of the species are anticipated to be of minor importance relative to the species' natural dispersal ability, which is both overland and via water ways (Courtenay & Williams, 2004).

Q. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name: RELEASE IN NATURE - Fishery in the wild (including game fishing)

(See also relevant sections under Introduction and Entry chapters)

Q. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	**intentional**	**CONFIDENCE**	**high**

Response: Anglers are well known to release sport fish to ‘enhance’ their fishery (Copp et al., 2005a). In the USA, this was demonstrated for *Channa* species (Courtenay & Williams, 2004).

Q. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: The movement of large fishes can range from a few specimens (Copp et al., 2003) to larger numbers (Copp et al., 2010), so such intentional releases will vary on a case-by-case basis.

Q. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Survival is estimated to be high as the transport of live sport fish is normally well organized, but even in bad conditions, *C. argus* would probably survive due to its broad tolerance to a wide range of environmental conditions and is extremely hardy (Cudmore & Mandrak, 2006). Owing to the species’ ability to air breathe, a consignment of *C. argus* was shipped from China to Canada without water and survived (Courtenay & Williams, 2004).

Q. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Assuming that the management practices would be even barely adequate at the locations where *C. argus* would be introduced as a sport fish, then the species would be likely to survive.

Q. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Intentional release of non-native fishes is illegal but still takes place and therefore goes unreported.

Q. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: *Channa argus* is highly tolerant of poor environmental conditions, and it is capable of upstream and downstream but also overland migrations (Courtenay & Williams, 2004; Lapointe et al., 2013) although movement out of water is more likely through marshy areas and during flooding (J. Hill, pers. comm.), so whether released into a suitable or unsuitable environment, the species has the ability to move until it finds a suitable habitat.

Q. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Response: Their functional terrestrial locomotor behaviours, combined with their emersion behaviour and efficient air-breathing capabilities, suggests that *C. argus* may be able to colonise new bodies of water via temporary overland movements (Bressman et al., 2019). But overland movement is very slow, and within-waterway migration apparently is seasonal and moderate (Lapointe et al., 2013). Overland migration is an ability to overcome local barriers to movement rather than a long-range migration ability (J. Hill, pers. comm.).

End of pathway assessment, repeat Q. 4.3 to 4.9. as necessary using separate identifiers.

Q. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in

relation to these pathways of spread?

RESPONSE	**very difficult**	**CONFIDENCE**	**medium**

Response: Once established in a river basin, whether in a still water or a water course, containment is likely to be difficult due to the species natural dispersal abilities and the many connections between basins in the risk assessment area.

Q. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	**slow**	**CONFIDENCE**	**medium**

Response: *C. argus* can tolerate a range of climates and could spread easily into many parts of the risk assessment area, but its natural dispersal ability and somewhat sedentary character suggest that the rate of spread would be slow under current climate conditions.

Q. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	**slow**	**CONFIDENCE**	medium

Response: *C. argus* can tolerate a range of climates and could spread easily into many parts of the risk assessment area, but its natural dispersal ability and somewhat sedentary character suggest that the rate of spread is unlikely to be affected by climate, so its spread would also be slow under future climate conditions.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1–5.5 relate to biodiversity and ecosystem impacts, 5.6–5.8 to impacts on ecosystem services, 5.9–5.13 to economic impact, 5.14–5.15 to social and human health impact, and 5.16–5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Q. A.7)

Biodiversity and ecosystem impacts

Q. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	**major**	**CONFIDENCE**	**high**

Comment: Gascho Landis et al. (2011) state that the total impact of the introduction of northern snakehead in the USA is still unknown but the potential for negative effects on native aquatic communities is large. Adult northern snakehead are highly piscivorous (Ling, 1977). Introductions of predatory fish can alter aquatic community structure and food webs through top-down mechanisms (Madenjian et al., 2002). Northern snakehead have broad environmental tolerances, and have the potential to significantly impact aquatic resources throughout North America (Herborg et al., 2007).

Channa argus is known to be ’highly predatory’ (Ling, 1977), with fish representing up to 33% of their diet (Courtenay & Williams 2004), spanning 17 species of prey fish, including loaches, breams, common carp *Cyprinus carpio*, and Eurasian perch *Perca fluviatilis* (Dukravets & Machulin, 1978). Investigations of *C. argus* diet in the U.S.A. have found that >97% of *C. argus* gut contents were fishes. In the Potomac River between 2004 and 2006 (Odenkirk & Owens, 2007) diet included banded killifish *Fundulus diaphanous*, white perch *Morone americana*, bluegill *Lepomis macrochirus*, pumpkinseed sunfish *Lepomis gibbosus*, which is also commonly consumed (Odenkirk & Owens, 2007). Other fish species in *C. argus* diet include goldfish *Carassius auratus*, gizzard shad *Dorosoma petenense*, American eel *Anguilla rostrata*, largemouth bass *Micropterus salmoides*, spottail shiner

Notropis hudsonias, eastern silvery minnow *Hybognathus regius*, mummichog *Fundulus heteroclitus*, channel catfish *Ictalurus punctatus*, green sunfish *Lepomis cyanellus*, and tessellated darter *Etheostoma olmstedi*.

Other than fishes, the species' diet includes crayfishes, dragonfly larvae, beetles, and frogs, amphibians and crustaceans (Courtenay & Williams 2004; Dolin 2003). *Channa argus* is considered to pose a threat through predation to threatened and endangered species, reduce biodiversity and to alter communities, especially those of naturally low species diversity (Courtenay & Williams, 2004).

Q. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	N/A	**CONFIDENCE**	high

Comment: At present, *C. argus* is not known to exist within the risk assessment area.

Q. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	**major**	**CONFIDENCE**	**medium**

Comment: If *C. argus* were to be introduced to the risk assessment area and released to the environment, then it is likely to exert adverse impact on biodiversity, especially in small water bodies of naturally-low species diversity (Courtenay & Williams, 2004). This is especially true of ponds, which are known to support disproportionately high aquatic biodiversity. Because no *Channa* species occurs naturally in Europe, there is no possibility of introduced snakeheads hybridising or interbreeding with native fishes.

Q. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds

and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	N/A	CONFIDENCE	-

Comment: At present, *C. argus* is not known to exist within the risk assessment area.

Q. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	major	CONFIDENCE	medium

Comment: The presence of *C. argus* is likely to have a major impact on the conservation status of both lakes and rivers, especially those with areas of dense aquatic vegetation and of naturally low species diversity and those containing endemic aquatic species (Courtenay & Williams, 2004).

Ecosystem Services impacts

Q. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comment: Owing to the voracious predatory nature of *C. argus*, the species poses a potential threat to aquatic ecosystem services associated with fisheries and aquaculture (Courtenay & Williams, 2004) through the reduction of fish or crustacean stocks.

The Department of Environmental Conservation of New York State (USA) warns that snakeheads have the potential to reduce or even eliminate native fish populations and alter aquatic communities. Municipalities which rely on tourist dollars from recreational fishing may suffer losses should northern snakeheads continue to invade their waters.[11]

Social consequences may exist should a population of snakehead become established, which negatively impacts commercial fisheries or other industries resulting in economic losses or reduction in quality of recreational usage of waterbodies (CABI, 2012).

Q. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Q. 5.6.

RESPONSE	**N/A**	**CONFIDENCE**	**-**

Comment: This species is not established within the RA area.

Q. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Q. 5.6.

RESPONSE	**minimal**	**CONFIDENCE**	**low**

Comment: No information has been found on this issue.

Economic impacts

Q. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere

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[11] https://www.dec.ny.gov/animals/45470.html

in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comment: An indication is provided by a modeling study that was conducted to determine if the expansion of invasive *C. argus* could negatively affect the population of a popular sport fish, the largemouth bass *Micropterus salmoides* in the Potomac River (Chesapeake Bay), U.S.A. (Love & Newhard, 2012). The distributions for both species were generated using catch records. *Channa argus* was not widely distributed during the study period and occurred mainly in upstream areas of tributaries. Many of these areas were moderately or highly suitable habitats for *M. salmoides*. Of sites where juvenile largemouth bass were collected, 10.6% were associated with *C. argus*. Using population modelling and measured predator–prey interactions, Love & Newhard (2012) determined that this level of co-occurrence would result in a 3.8% reduction in *M. salmoides* population size. This prediction is consistent with observations that indicate there has not been a negative trend in the *M. salmoides* fishery. As co-occurrence was increased in the model, however, the negative impact of *C. argus* on largemouth bass monotonically increased. The time required for such increases in *C. argus* distribution could not be determined, but if *C. argus* continued to expand its range in the absence of control measures, then the population model, with its assumptions, predicted a 35.5% reduction in the abundance of *M. salmoides.*

Q. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the risk assessment area these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**N/A**	**CONFIDENCE**	**-**

Comments: *Channa argus* is not currently present in the RA area.

Q. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Q. 5.10.

RESPONSE	**Moderate**	**CONFIDENCE**	**low**

Comments: The potential economic cost/loss due to damage if *C. argus* were to establish in the risk assessment area would depend upon the extent of the species spread, the success or not of efforts to extirpate the species, and the locations where it invades (i.e. those of conservation value being of particular concern).

Q. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	N/A	**CONFIDENCE**	**-**

Comments: *Channa argus* is not currently present in the RA area.

Q. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Q. 5.12.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comments: The economic costs of eradication of *C. argus* could be relatively modest or very high, depending upon the extent of the species' spread, the size of the water bodies it invades, etc. As mentioned above, the cost of *C. argus* eradication from a small pond in Crofton, Maryland (U.S.A.), was estimated to be $110k USD (≈ €100k), encompassing personnel time for planning meetings, field application of the piscicide, and disposal of the dead fish (Courtenay & Williams, 2004). Costs of eradication would increase with increasing larger waterbody size (e.g. Britton et al., 2010, 2011), but on average £20K GBP per hectare (≈ €22k/ha) (Britton et al., 2008).

Social and human health impacts

Q. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries,

if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**N/A**	**CONFIDENCE**	**-**

Comments: *Channa argus* is not currently present in the risk assessment area.

Q. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	moderate	**CONFIDENCE**	low

Comments: As mentioned here above, if *C. argus* is introduced to the risk assessment area and released to the environment, then the potential risks to social/human health appear limited, based on current knowledge. There is one snakehead species, the chevron snakehead *Channa striata*, that has been found to be an intermediate host of the helminth parasite *Gnathostoma spinigerum*, which causes gnathostomiasis, a disease which may be transmitted to humans (Cudmore & Mandrak, 2006). The fact that one *Channa* species has been shown as a carrier indicates that there are other species that could present a similar threat to human health, though this has yet to be investigated (Courtenay & Williams, 2004). No other information on potential threats to social or human health were found.

Other impacts

Q. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	major	**CONFIDENCE**	Medium

Comments: Potential to transfer pathogens (parasites, diseases) is largely unknown. A paper by Chiba et al. (1989) mentions that *C. argus* introduced in 1923/24 from Korea had parasites but provides no further detail.

Nevertheless, all snakehead species are hosts to at least several species of parasite. See table below from Courtenay & Williams (2004)

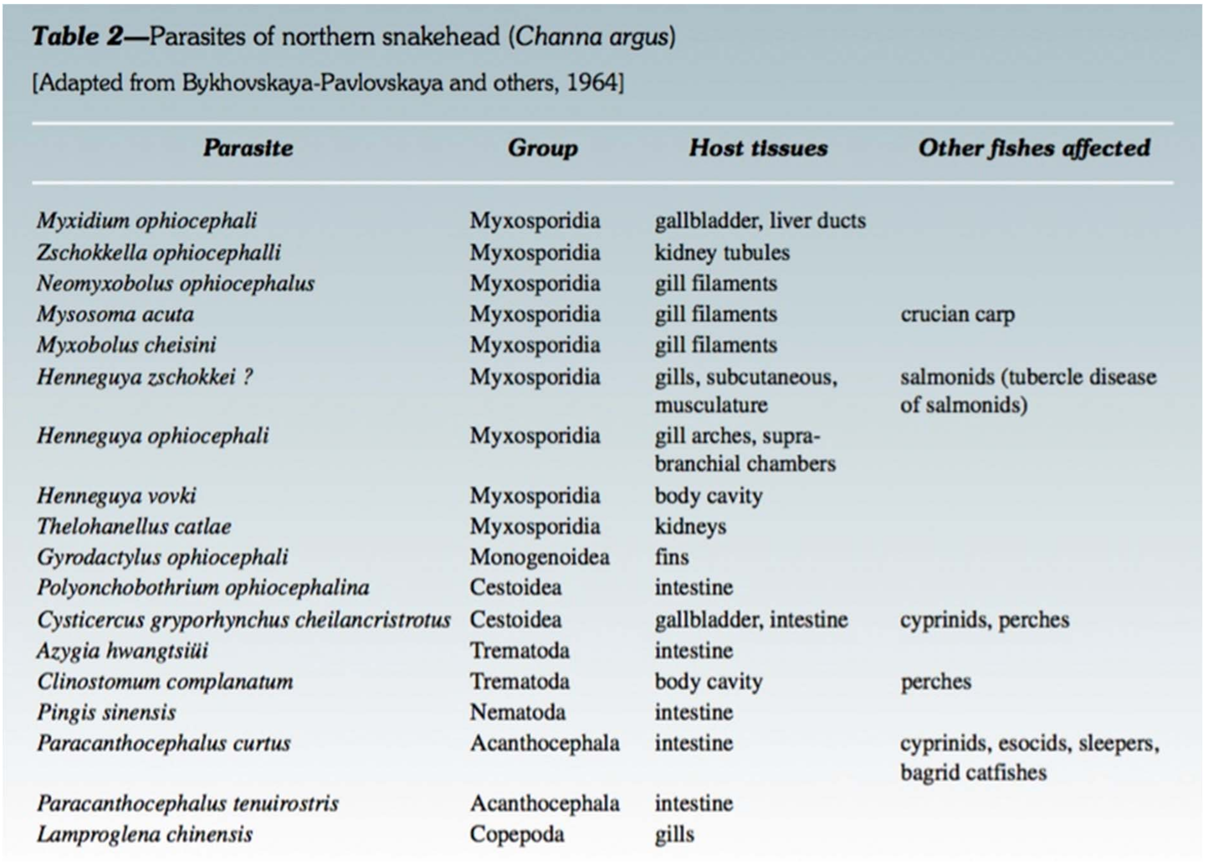

Table 2—Parasites of northern snakehead (*Channa argus*)

[Adapted from Bykhovskaya-Pavlovskaya and others, 1964]

Parasite	***Group***	***Host tissues***	***Other fishes affected***
Myxidium ophiocephali	Myxosporidia	gallbladder, liver ducts	
Zschokkella ophiocephalli	Myxosporidia	kidney tubules	
Neomyxobolus ophiocephalus	Myxosporidia	gill filaments	
Mysosoma acuta	Myxosporidia	gill filaments	crucian carp
Myxobolus cheisini	Myxosporidia	gill filaments	
Henneguya zschokkei ?	Myxosporidia	gills, subcutaneous, musculature	salmonids (tubercle disease of salmonids)
Henneguya ophiocephali	Myxosporidia	gill arches, supra-branchial chambers	
Henneguya vovki	Myxosporidia	body cavity	
Thelohanellus catlae	Myxosporidia	kidneys	
Gyrodactylus ophiocephali	Monogenoidea	fins	
Polyonchobothrium ophiocephalina	Cestoidea	intestine	
Cysticercus gryporhynchus cheilancristrotus	Cestoidea	gallbladder, intestine	cyprinids, perches
Azygia hwangtsiüi	Trematoda	intestine	
Clinostomum complanatum	Trematoda	body cavity	perches
Pingis sinensis	Nematoda	intestine	
Paracanthocephalus curtus	Acanthocephala	intestine	cyprinids, esocids, sleepers, bagrid catfishes
Paracanthocephalus tenuirostris	Acanthocephala	intestine	
Lamproglena chinensis	Copepoda	gills	

At least two snakehead species used in intense aquaculture, *C. punctata* and *C. striata*, are susceptible to epizootic ulcerative syndrome (EUS), a disease believed to be caused by several species of bacteria, a fungus, and perhaps a retrovirus. Li et al. (2019) also describe the effects of this disease on the hybrid snakehead (*Channa maculata*♀ × *Channa argus*♂) so it is very likely that EUS can also affect *C. argus* itself. The EUS causes high mortality in these fishes but it is not specific to snakeheads and has affected other fishes, such as clariid catfishes, bagrid catfishes, two cyprinid genera, mastacembalid eels, a nandid fish in India, and giant gourami and climbing perch in Thailand. The EUS involves several pathogens (Courtenay & Williams, 2004), including motile aeromonad bacteria (for example, *Aeromonas hydrophila, A. caviae, Pseudomonas fluorescens*), a fungus, *Aphanomyces invadans*, which is considered a primary pathogen, and perhaps a rhabdovirus. Another bacterium, *Aquaspirillum* sp. has also been implicated. There have been no studies undertaken to examine transfer of parasites or diseases to native North American fishes (Courtenay & Williams, 2004), and the same appears to be true for fishes native to the risk assessment area.

Q. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	**minimal**	**CONFIDENCE**	**low**

Comments: No other impacts have been recorded in the species' non-native range, which suggests if any exist, they are of minimal magnitude.

Q. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	**major**	**CONFIDENCE**	**low**

Comments: In view of the previously described potential impacts, and the unlikely natural control on this voracious predator fish, the anticipated impacts would still be major.

Q. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	**major**	**CONFIDENCE**	**medium**

Q. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	**major**	**CONFIDENCE**	**medium**

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction*	**unlikely**	**medium**	Currently, *C. argus* is not present in the risk assessment area and no active introduction pathways are known. *Channa argus* is available for sale on the internet and, although not very popular with aquarists, could still be imported by some people. Other pathways (as described for the USA and Canada e.g. live food, angling, prayer animal release) are not taken into account in this risk assessment as estimated to be non-existing in the risk assessment area.
Summarise Entry*	**moderately likely**	**medium**	Because snakeheads represent only a very minor component of aquarium fish trade in the risk assessment area, the illegal release or dumping of this species in the environment will never encompass large numbers of individuals. However, *C. argus* specimens would have a high chance of being dumped because of their highly predacious nature and the significant costs associated with feeding and housing this species. They also are not very colourful and rapidly attain very large sizes.
Summarise Establishment*	**very likely**	**high**	Appropriate habitats and climate are found throughout most of the risk assessment area and *C. argus* and its congeners have invasion histories characterised by successful establishment outside their native ranges, which is facilitated by their high tolerance of poor water quality conditions.
Summarise Spread*	**moderate**	**medium**	*Channa argus* is tolerant of a wide range of environmental conditions, it is able to migrate overland and undertakes modest seasonal migrations, so its rate of spread in the risk assessment area is likely to slow unless translocated by humans, which could be for sport fishing reasons, for example.

Summarise Impact*	**major**	**high**	The introduction of a small number (<5) of *C. argus* specimens into an isolated spring habitat could result in extinction through predation of endemic spring-adapted fishes or crustaceans (Courtenay & Williams, 2004), with competition for food resources also considered high. This species would present a potential economic threat to wild fish stocks and to fish culture interests, especially if this species enters culture facilities from adjacent waters (Courtenay & Williams, 2004). These impacts would have both ecosystem services and socio-economic consequences.
Conclusion of the risk assessment (overall risk)	**High**	**medium**	Although the introduction of this species is considered unlikely, if this species was to find its way in the risk assessment area, then it is likely to spread and exert major impacts.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	-	-	Yes	Yes	-
Belgium	-	-	Yes	Yes	-
Bulgaria	-	-	Yes	Yes	-
Croatia	-	-	Yes	Yes	-
Cyprus	-	-	Yes	Yes	-
Czech Republic	Yes	-	Yes	Yes	-
Denmark	-	-	Yes	Yes	-
Estonia	-	-	Yes	Yes	-
Finland	-	-	-	Yes	-
France	-	-	Yes	Yes	-
Germany	-	-	Yes	Yes	-
Greece	-	-	Yes	Yes	-
Hungary	-	-	Yes	Yes	-
Ireland	-	-	Yes	Yes	-
Italy	-	-	Yes	Yes	-
Latvia	-	-	Yes	Yes	-
Lithuania	-	-	Yes	Yes	-
Luxembourg	-	-	Yes	Yes	-
Malta	-	-	Yes	Yes	-
Netherlands	-	-	Yes	Yes	-
Poland	-	-	Yes	Yes	-
Portugal	-	-	Yes	Yes	-
Romania	-	-	Yes	Yes	-
Slovakia	-	-	Yes	Yes	-
Slovenia	-	-	Yes	Yes	-
Spain	-	-	Yes	Yes	-
Sweden	-	-	-	Yes	-
United Kingdom	-	-	Yes	Yes	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	-	-	-
Atlantic	-	-	Yes	Yes	-
Black Sea	-	-	Yes	Yes	-
Boreal	-	-	Yes	Yes	-
Continental	-	-	Yes	Yes	-
Mediterranean	-	-	Yes	Yes	-
Pannonian	Yes 1956	-	Yes	Yes	-
Steppic	-	-	Yes	Yes	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea					
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast				-	
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea					
Aegean-Levantine Sea					
Ionian Sea and the Central Mediterranean Sea					
Western Mediterranean Sea					

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[12]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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[12] Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition,*

			predations, spread of disease etc.)
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[13]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*

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[13] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*

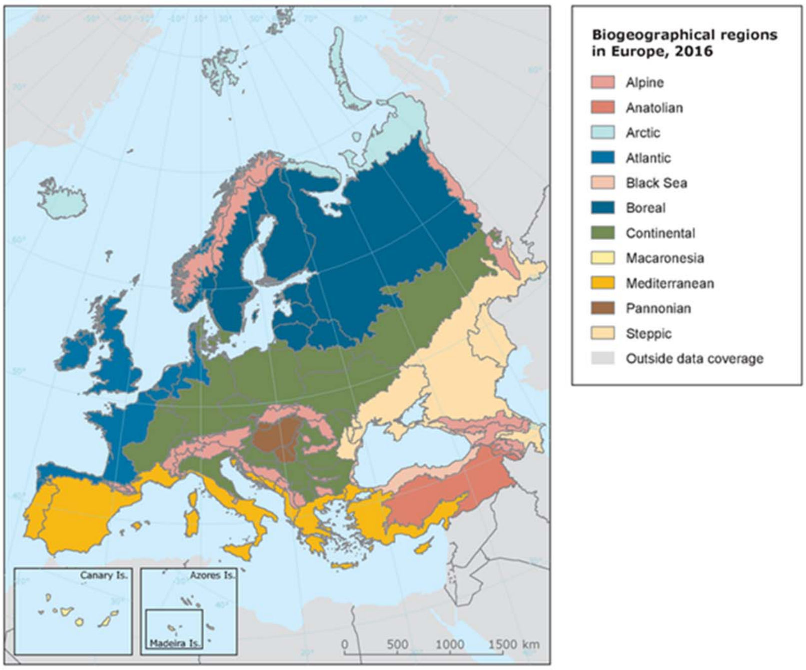
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

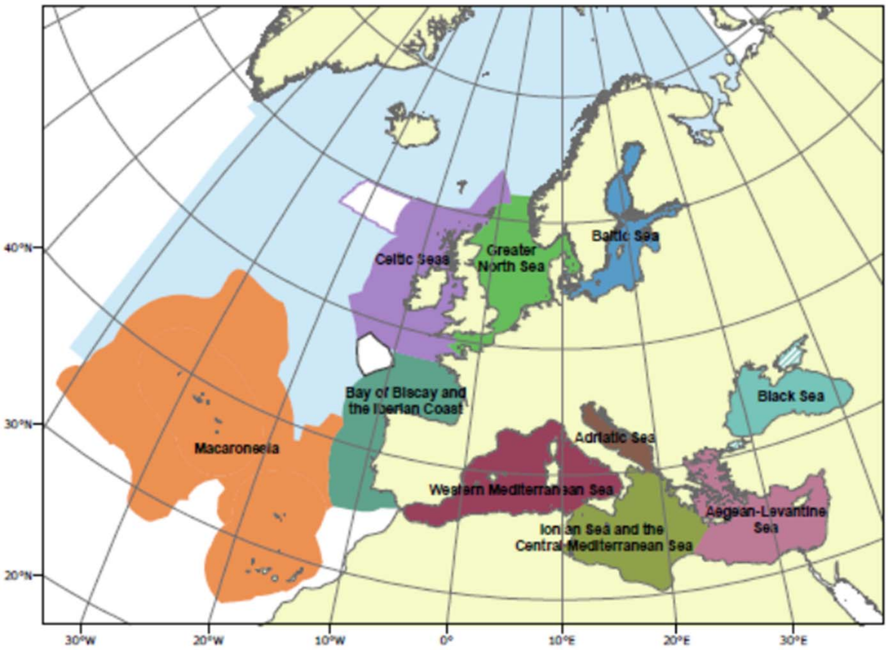
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	*Channa argus* (Cantor, 1842)
Species (common name)	**Northern snakehead**
Author(s)	**H. Verreycken, L.R. Aislabie, G.H. Copp**
Date Completed	**23 October 2019**
Reviewer	**John S. Odenkirk, Jeffrey E. Hill**

Summary [1]

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

An important prevention technique used by countries inside and outside of the RA area is legislation (prohibition on the import, keeping and trade of *Channa argus*). Thereafter, the most cost-effective means of preventing a species' introduction, such as that of *Channa argus*, is to raise public awareness of the threats posed by the species and the management of the vectors by which the species could be introduced to the EU and its entry into the environment. Public awareness and education may not be expensive tasks, but a better understanding of threats posed by invasive species will help motivate people and increase the likelihood that they will not release the animals they keep as pets into the environment and they will subsequently be more likely to report invasive species when they encounter them.

Early detection of the species within the RA area is very hard due to the areas this species inhabits. Additional reporting from recreational fishermen and commercial fishing vessels of new findings of this fish would benefit the targeted monitoring for the species. Although river surveys would be a continuous way of early detection, it can be a very expensive measure and it is not guaranteed to detect the species. A potentially cost-effective means of enhancing the detection of undesirable, prohibited non-native species is the use of environmental DNA (eDNA) approaches.

Rapid eradication of any species will depend on where and at what stage the species is first reported. The potential to eradicate or control non-native fishes depends on the type of aquatic environment in which they are found, their potential dispersal from that location, and whether or not they have begun to establish a self-sustaining population. In general, fishes can be particularly difficult, and in some cases practically impossible, to extirpate from water courses and water bodies. In small enclosed water bodies the use of drain-down, mechanical removal (e.g. using traps, nets or electrofishing), and piscicides (rotenone) may be effective in eradicating populations, with the level of difficulty (or impossibility) of eradication increasing with the size, complexity and conservation value of the water body or water course inhabited by the species targeted for eradication.

In cases where eradication is impossible, mechanical removal, e.g. by electrofishing and use of other fishing gear, may be successful to contain and manage invasive fish populations. Targeted angling for the species can also be used as part of the removal and/or control exercise, which in some cases of on-going control may sustain a small fishery.

More research and better understanding of invasive fish species could reveal other ways for their eradication or control.

The limited amount of literature available on *C. argus* eradication programmes and their associated costs derive mainly from the U.S.A.

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)**[3]	**Level of confidence**[4]
Methods to achieve prevention [5]	**Managing the pathways by a ban on importing, keeping and trading of *Channa argus*, including the aquarium trade to reduce the risk of intentional introductions.** *Channa argus*, is not yet present in the RA area. Four possible introduction vectors have been identified for the U.S.A. and Canada: intentional introduction for aquaculture, intentional introduction for the pet trade, release for angling purposes, and	A ban of live sale of *C. argus* would be an effective means of limiting the risk of introduction of the species for the aquarium trade pathway. There is no information available about the costs and the equipment or infrastructure that may be required to implement this measure, but it is widely accepted that prevention is more cost effective than management of the entry or establishment of such a species group (Savior, 2016). This measure would need to include the provision and training of administration and staff to enforce the regulations.	Medium

	release as part of a religious ceremony (Cudmore & Mandrak, 2006). Only two of these vectors are considered relevant to the EU: introduction for aquaculture and for the pet trade. The adoption and enforcement of appropriate legislation and codes of best practice could reduce the likelihood of introduction. A ban on the import and sale of live *C. argus* would be an effective means of preventing the species introduction to the EU, thus rendering the species unavailable to the aquaculture and pet trade sectors.		
	Increasing public awareness, including education and training to reduce the risk of intentional, and un-intentional introductions: Species of the Genus *Channa* are probably only being imported in the RA area for the aquarium trade, although the larger-bodied species of the Genus, e.g. *C. argus*, are not favoured for the aquarium or water garden trade as they are	Campaigns to educate people and increase awareness on IAS are an effective way to curb (il)legal introductions, especially those targeted at specific sectors. Public awareness campaigns, however, do need to be maintained so they do not drop out of the collective consciousness, but are also renewed periodically to avoid fatigue. Ideally the development of awareness raising campaigns and educational materials needs to be done for each member state, guided by scientific expertise and co-ordinated by an "education committee" or a similar initiative. Resources required, and associated costs, are dependent upon the activities and materials developed, but	Low

	not very colourful and rapidly attain very large sizes (Courtenay and Williams, 2004). Other species of the genus *Channa*, however, are clearly more introduced in several EU member states for the aquarium trade and are for sale in aquarium shops and on the internet. It is important to raise awareness about the possible consequences of releasing the coolwater-adapted, potentially invasive, species such as *C. argus* into open waters.	maybe include media campaigns, websites, marketing materials, or outreach training and education schemes (Roy et al., 2018). Costs of campaigns to increase awareness are estimated to be low to medium (€50–200K/year) on an EU scale.	
Methods to achieve eradication [6]	**Effective surveillance and reporting:** *Channa argus* is a readily identifiable species although it may be confused with species from the same genus. Channids are difficult to identify especially when mature, and their taxonomy is not fully settled (Zogaris, 2017). Effective eradication is most likely to be achieved when new invasions are quickly reported. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the	Trawl nets, fyke nets, traps, and electrofishing can be used to detect and monitor for non-native fishes in the RA area, even if not always effective at low density (Britton et al., 2011) in which case environmental DNA approaches can be used (e.g. Dougherty et al., 2016; Davison et al., 2017, 2019), including in large lakes (Larson et al., 2017). Environmental DNA based monitoring could be considered also for the entire RA area. Citizen science could be promoted to monitor the possible introduction and spread of the species. If dedicated monitoring for *C. argus* is not possible, then monitoring for this species can be incorporated in already running monitoring programmes e.g. for the Water Framework Directive. Although the above mentioned tools may be effective in	Low

	species can become established. Post-eradication detection can also be undertaken to determine whether or not an eradication action has been successful. A simple and clear identification sheet could be drafted and distributed to different stakeholders (e.g. anglers, aquarists) to increase the probability of an early detection and rapid response	early detection, eradication of *C. argus* after first find can only be effective when the detected infestation is low, with the potential feasibility and effectiveness of the eradication dependent on the size and type of infested water, still waters being easier than water courses, and feasibility decreases (and costs increase) with increasing size of the water needing eradication action. In large riverine systems, which are a typical habitat of *C. argus*, eradication may be impossible (see also below). The costs of dedicated surveillance and monitoring and subsequent removal of invasive fish are estimated to be medium (€200K–1M for five years) for the RA area. Eradiation costs per surface area have been estimated in the U.K. as on average £20K GBP per hectare (≈ €22k/ha) (Britton et al., 2008)	
	Use of piscicide (chemical removal): a piscicide can be used to kill newly-detected populations in smaller areas such as ponds, drainable larger water bodies (e.g. reservoirs), or small water courses. There may be legal constraints as e.g. Rotenone was withdrawn from use in the European Union in 2007 (Schapira, 2010), but is still used in the U.K. (Britton et al., 2008, 2010; UK Environment Agency, 2014).	*Channa argus* can be killed by rotenone or other piscicides, even although it is a obligate air breather (John Odenkirk, pers.comm.). However, it would be difficult (if not impossible) to make an effective eradication in large rivers. The potential to eradicate or control snakehead populations depends on dispersal location and the level of establishment. If broadly dispersed in large lakes or river systems, then eradication or control would likely be impossible. Management options for population control within smaller water bodies are dependent upon the amount of aquatic vegetation, accessibility of the waterbody, and the effectiveness of the control techniques employed (Hoffman, 2002). As such, in 2002, the first established population found in the USA was in a Maryland	High

		retention pond, which has since been treated with rotenone, eradicating the population (Orrell & Weigt, 2005). Effective removal strategies for established populations of snakehead must rely on several methods to eradicate the species from non-native waterbodies due to their uncommon biological attributes. Chemical removal using piscicides, such as rotenone, which acts to impede oxygen availability to fish may not be very effective against *C. argus* due to its ability for air breathing and would likely only result in the removal of non-target species. Recent research, however, has learnt that some of these special features (overland movement, sensitivity to piscicides, …) of *C. argus* are overstated, so snakeheads may not be the highly invasive species they were once feared to be (Odenkirk, 2018). Eradiation costs can be high. The costs per surface area have been estimated in the U.K. as on average £20K GBP per hectare (≈ €22k/ha) (Britton et al., 2008)	
	Mechanical removal: Mechanical removal of *Channa* species can be done by gill netting, seine netting, perhaps by fyke/hoop nets, and electrofishing. Protocols for removal are well developed for a wide variety of fishes including predatory fishes similar to *Channa* species (e.g West et al., 2007) but	Eradication campaigns of *C. argus* with mechanical removal techniques may be most effective during particular parts of the year when many specimens gather and make removal applications more accurately targeted. In temperate waters, in the spring spawning season prior to juvenile dispersal (Jiao et al., 2009) some species such as *C. argus* are least mobile (Lapointe et al., 2010). For *C. argus* spawning occurs between May and July (CABI 2019). Habitat selection is the strongest during the spawning season, suggesting that locations likely to harbour *C. argus* can be most easily	Medium

	electrofishing is preferred because it has the least amount of by-catch and damage to native fish populations (Mueller, 2005). Angling is also locally effective in removing large numbers of these predatory fishes (Savior, 2016).	targeted at this time of year. There is a pronounced upstream migration (even in tidal systems) pre-spawn – usually late April to early May along the mid-Atlantic U.S. Water temperature is usually between 15 and 20 °C during this time. Northern snakeheads are excellent migrants and can jump and negotiate barriers better than many anadromous species. They will accumulate below dams and other impediments to upstream migration during this time, but will dissipate after several weeks (J. Odenkirk, pers. comm.). Mechanical removal may be the only way to treat a system where chemical piscicides cannot be applied. Angling and increased fishing effort by amateurs could also be part of the overfishing effort (Savior, 2016). Finally, the possibility of combining mechanical removal with drastic habitat alteration may also help or increase the synergistic pressures on an isolated population of large predatory fishes such as these. This approach is case-specific and would involve draining reservoirs or altering water levels to increase fish density and localise them in an enclosed water body (Zogaris, 2017). However, this measure will be more effective to manage and contain invasive fish populations than to eradicate a population of *C. argus*.	
Methods to achieve management [7]	**Raising awareness:** Raising public awareness of the risks posed by invasive non-native species in general and *C. argus* in particular may diminish the chance of new introductions after eradication/management	Costs for outreach and production of leaflets can be high when applied across a large community, such as for the EU.	Medium

	of an invasive species. The production of targeted publicity and identification material is needed.		
	The **above methods** described to support eradication can also be used to manage existing *C. argus* populations.	See above	See above
	Reducing risks of further dispersal	Dedicated monitoring (e.g. fyke nets, trawl nets and especially electrofishing but also eDNA) of water courses and water bodies is necessary to detect the presence of non-native species and to ensure that these waters are not recolonised by the species after eradication. In parallel, to prevent further spread and new introductions, a prohibition on the keeping and release (especially for aquarists) of regulated species should be enforced. Also, stringent procedures should be put in place to check imported and within-EU consignments of fish intended for aquarium trade. Depending on the area that has to be monitored, management costs can be from medium to very high (from <€5k to > €1M).	Medium
	Further research	Additional research of *C. argus* is needed but would be expensive. Studies are needed into the extent of trade of *Channa* species in Europe, as well as the species adaptability to EU environments (e.g. Frank, 1970). These are currently scarce, and perhaps the presence of the species in the aquarium and aquaculture market appears to be overlooked despite some reports of the species in the wild, even if dead, e.g. giant snakehead *Channa micropeltes* (Zięba et al.,	Medium

		2010). More information is required concerning the imports of *Channa* species into the EU Member States, and their status in the aquarium, aquaculture and the internet trade (Zogaris, 2017).	

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; this is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: *Ameiurus melas* (Rafinesque, 1820)
Common name: black bullhead

Authors of the assessment:

- *Luke Aislabie, Cefas, Lowestoft, U.K.*
- *Hugo Verreycken, Research Institute for Nature and Forest (INBO), Brussels, Belgium*
- *Gordon H. Copp, Cefas, Lowestoft, U.K*

Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: *Ján Koščo, Department of Ecology, University of Prešov, Slovakia*

Peer review 2: *Elena Tricarico, University of Florence, Florence (UNIFI)*

Date of completion: 23 October 2019

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: *Ameiurus melas* belongs to the genus *Ameiurus* (Rafinesque, 1820), which is part of the Siluriformes (catfishes), Ictaluridae (Gill, 1861), North American freshwater catfishes (Froese & Pauly, 2019):

Kingdom Animalia

Phylum Chordata

Class Actinopterygii

Order Siluriformes

Family Ictaluridae

Ictaluridae consists of eight genera (one extinct) and 67 species of which 51 are extant (12 with fossil records) and 16 extinct (Arce-H. et al., 2016). Monophyly of living Ictaluridae is well supported by molecular data analysed using parsimony and model-based methods. These analyses found further support for the monophyly of the genus *Ameiurus*. This genus is represented by 16 species of which nine are fossils (Arce-H. et al., 2016).

According to the Integrated Taxonomic Information System (www.itis.org) *Ameiurus* spp. comprises of the following species:

- *Ameiurus brunneus* (Jordan, 1877) – snail bullhead
- *Ameiurus catus* (Linnaeus, 1758) – white catfish, white bullhead
- *Ameiurus melas* (Rafinesque, 1820) – black bullhead
- *Ameiurus natalis* (Lesueur, 1819) – yellow bullhead
- *Ameiurus nebulosus* (Lesueur, 1819) – brown bullhead

- *Ameiurus platycephalus* (Girard, 1859) – flat bullhead
- *Ameiurus serracanthus* (Yerger and Relyea, 1968) – spotted bullhead

Synonyms (non-valid) for *A. melas* are *Silurus melas*, *Ictalurus melas* and *Ictalurus melas melas*. Common name for *A. melas* is black bullhead but also used are black catfish, yellow belly bullhead or hornedpout (Froese & Pauly, 2019).

Ictalurid catfish species (also referred to as bullheads) have an adipose fin between their dorsal and tail fins. They have a rounded tail which will help to distinguish them from small channel catfish, *Ictalurus punctatus*, that have a forked tail. Ictalurid catfishes have no scales, their bodies are covered with taste buds, and will be very slippery to handle. Finally, ictalurid catfishes have a single, sharp spine in the dorsal and pectoral fins. Like other members of the Ictaluridae, black bullhead also has barbels (‘whiskers’) under their chin that help them locate food (Scott and Crossman, 1973).

In the risk assessment area, currently only brown *A. nebulosus* and black bullhead *A. melas* are established. Other species (white and yellow bullhead) were only recorded very occasionally. There are a number of reports of the introduction of *A. natalis* (yellow bullhead) into Italy (Holčík, 1991). However, there is no reliable evidence for this (Godard, 2015). Confirmed presence exists for *Ameiurus catus* (white catfish) only in the UK (Britton and Davies, 2006; Zięba et al., 2010).

Ameiurus melas (black bullhead) is known to hybridise naturally with their close congeners *A. nebulosus* and *A. natalis* (Hunnicutt et al., 2005).

The species in the genus are sometimes very difficult to distinguish from each other, especially *A. melas* and *A. nebulosus* (Wheeler, 1978).

One of the main distinguishing features that distinguish *A. melas* and *A. nebulosus* is that the *A. melas* has a weak serration on the trailing edge of the pectoral spines; whereas for *A. nebulosus*, the pectoral spine edge comprises regular saw-like barbs. The colour pattern also varies with *A. melas* being mainly dark, whereas *A. nebulosus* is usually mottled, but may be solid also (CABI, 2019). An important feature to distinguish *A. melas* and *A. nebulosus* is the colouration of the caudal and anal fin membrane (Decru and Snoeks, 2011): *A. melas* always has a black-and-white radiation on the caudal and anal fins, whereas *A. nebulosus* clearly does not have this. *Ameiurus melas* has lightly coloured fin rays with the tissue between the fin rays always dark, which causes this black-and-white radiation. For *A. nebulosus* the entire fins are rather light in colour.

Confusion between species could be possible, so identification of other species in the genus as *A. melas* or *A. nebulosus* cannot be ignored (Lenhardt et al., 2011).

The known common names of *Ameiurus melas* in European languages other than English are the following: NL: zwarte Amerikaanse dwergmeerval, DK: sort dværgmalle, PL: sumik czarny, DE: Schwarzer Katzenwels, FR: Poisson-chat, IT: Pesce gatto, ES: Pez gato negro.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed,

including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

There are two spcies of the genus *Ameiurus* in the risk assessment area, the black and the brown bullhead (Wheeler, 1978). There is a high degree of morphological similarity between *A. nebulosus* and *A. melas*. Differences have been mentioned in the previous question.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: *Ameiurus melas* has been ranked in several European countries/regions as representing a “medium” or “high” risk of being invasive using the fish invasiveness screening kit (FISK: Copp et al.,2009).

Ameiurus melas was classified as “high” risk for England & Wales (Copp et al. (2009).

Puntila et al. (2013) concluded that the risk of invasion for southern Finland was “medium”.

In Balkans Region, this species has a “medium-high” risk to become invasive (Simonović et al., 2013).

Piria et al., (2016) categorised *A. melas* as “high” risk of being invasive for Croatia and Slovenia.

The species is categorised as “high” risk of being invasive in the drainage basin of Lake Balaton, Hungary (Ferincz et al., 2016).

For the Iberian Peninsula, Almeida et al. (2013) classified the species as “very high” risk of being invasive.

Tarkan et al. (2014) categorised the species as “high” risk for Turkey, which is part of the frontier between Asia and Europe (Anatolia and Thrace).

Outside Europe, this species was identified as a potentially high-risk noxious species as a result of a rapid risk assessment approach that was developed in Australia (Moore et al., 2010). The Department of Fisheries of the Government of Western Australia (2013) included this species in State’s Noxious Fish List.

The species has been translocated within its native North America, introduced into the Pacific Northwest and reported for British Columbia, Canada, in the mid-1980s (Forbes and Flook, 1985) but no detailed risk screening could be found for that area.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: Native to the Great Lakes, Hudson Bay, and Mississippi River basins in most of the eastern and central United States and adjacent southern Canada and northern Mexico, south to the Gulf Coast (Gulf Coast drainages from Mobile Bay in Georgia and Alabama to northern Mexico) (Page and Burr, 1991); apparently not native to the Atlantic Slope (Fuller and Neilson, 2017).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Introduced widely outside the native range (Rose, 2006). Apart from Europe, it has been introduced also in Chile (Iriarte et al., 2005; Froese and Pauly, 2019), Mexico (Page and Burr, 1991; Froese and Pauly, 2019), to many states in the USA, and western parts of Canada (Scott & Crossman, 1973; Forbes and Flook, 1985).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a): Atlantic, Boreal, Mediterranean, Pannonian

Response (6b): Atlantic, Boreal, Mediterranean, Pannonian

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Following Climatch (Peel et al. 2007) all biogeographic regions, except probably the Alpine region, of the risk assessment area have at current climate more or less suitable climate for establishment of *A. melas*.

Atlantic Region

Black Sea Region

Boreal Region

Continental region

Mediterranean region

Pannonian Region

Steppic Region

Response (7b): Britton et al. (2010a) ran a comparison of mean Climatch scores between 2009 and 2050 for *A. melas* in the UK. *Ameiurus melas* has an increased climate match with the source region in 2050 when compared with 2009. This species is likely to benefit from climate warming in England and Wales, this prediction was then tested using water temperature modeling. One can expect that similar benefit is true for regions between 50° and 55° N as modeled by Britton et al. (2010a).

Atlantic Region

Black Sea Region

Boreal Region

Continental biogeographical region

Mediterranean biogeographical region

Pannonian Region

Steppic Region

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):

- Austria (Wiesner et al., 2010) – first record unknown
- Belgium (Verreycken et al. 2007)) – first record *ca*. 1882
- Croatia (Jelić et al., 2010;) – first record *ca.* 2005
- Czech Republic (Hartvich and Lusk, 2006) –first record *ca.* 2003
- France (Holčík, 1991; Rutkayová et al., 2013)) – first record *ca.* 1885

- Germany (Wolter et al., 2000; Wiesner et al., 2010)) – first record *ca.* 1885
- Hungary (Bódis et al., 2012)) – first record *ca.* 1985
- Italy (Holčík, 1991; Rutkayová et al., 2013)) – first record *ca.* 1900
- Poland (Nowak et al., 2010a; Holčík, 1991; Rutkayová et al., 2013) – first record *ca.* 1900
- Portugal (Ribeiro et al., 2006) – first record *ca.* 2002
- Romania (Wilhelm, 1998; Gaviloaie and Falka, 2006) – first record *ca.* 1968
- Slovakia (Koščo et al., 2004; Rutkayová et al., 2013) – first record *ca.* 1999
- Slovenia (Piria et al., 2016) – first record unknown
- Spain (Elvira, 1984; Copp et al., 2016)) – first record *ca.* 1950
- Sweden, recorded in 2014 at one location and successfully eradicated in 2015 (Brockmark, 2015; GBIF Secretariat, 2018)
- The Netherlands (Holčík, 1991; Rutkayová et al., 2013)) – first record *ca.* 1900
- UK (Holčík, 1991; Rutkayová et a., 2013) – first record *ca.* 1880

Response (8b): There are established populations in 15 EU Member States. Most introductions ended in established populations but establishment dates are almost never published. In general, establishment date is not so much different from date of first record (see 8a):

- Austria (Wiesner et al., 2010)
- Croatia (Ćaleta et al., 2011)
- Czech Republic (Musil et al., 2008)
- France (Thiero Yatabary, 1981; Copp, 1989; Keith et al., 2011; Cucherousset et al., 2006a)
- Germany (Arnold, 1990; Wolter and Röhr, 2010)
- Hungary (Pintér, 1991; Bódis et al., 2012)
- Italy (Bianco, 1998; Copp et al., 2016; Pedicillo et al., 2008)
- Poland (Nowak et al., 2010a, 2010b; Grabowska, 2010)
- Portugal (Almaça, 1995; Gante and Santos, 2002; Ribeiro et al., 2006)
- Romania (Wilhelm, 1998; Copp et al., 2005a; Gaviloaie and Falka, 2006)
- Slovakia (Koščo et al., 2010)
- Slovenia (Piria et al., 2016)
- Spain (Miranda et al., 2010, De Miguel et al., 2014)

- The Netherlands (Verreycken et al., 2010; NDFF and RAVON/ANEMOON, 2018)
- UK (Lever, 1977; Wheeler, 1978; Copp et al., 2016) but the only confirmed population has been eradicated (UK Environment Agency, 2014)

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9–2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): This species could probably establish in all the EU Member States, given its broad native range (Scott and Crossman, 1973), also in the Member States which currently are not known to have established populations:

- Austria (Wiesner et al., 2010)
- Belgium (Verreycken et al., 2007)
- Bulgaria
- Croatia (Ćaleta et al., 2011)
- Cyprus
- Czech Republic (Musil et al., 2008)
- Denmark
- Estonia
- France (Copp, 1989; Keith et al., 2011; Copp et al., 2016; Cucherousset et al., 2006a)

- Finland
- Germany (Arnold, 1990; Wolter and Röhr, 2010)
- Greece (Barbieri et al., 2015)
- Hungary (Pintér, 1991; Bódis et al., 2012)
- Italy (Bianco, 1998; Pedicillo et al., 2008; Copp et al., 2016)
- Latvia
- Lithuania
- Luxembourg (Copp et al., 2016)
- Malta
- Poland (Nowak et al., 2010a, 2010b)
- Portugal (Almaça, 1995; Gante and Santos, 2002; Ribeiro et al., 2006)
- Romania (Wilhelm, 1998; Copp et al., 2005a; Gaviloaie and Falka, 2006)
- Slovakia (Koščo et al., 2010; Copp et al., 2016)
- Slovenia (Piria et al., 2016)
- Spain (Miranda et al., 2010, De Miguel et al., 2014; Copp et al., 2016)
- Sweden
- The Netherlands (Verreycken et al., 2007, 2010; NDFF and RAVON/ANEMOON, 2018)
- UK (Lever, 1977; Wheeler, 1979; Copp et al., 2016)

Response (9b): Same as 9a, see question 7b for establishment under climate change.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:

Nearly all risk assessments (see A3) rank *A. melas* as 'medium' or 'high' risk of being (or becoming) invasive in the member states of the risk assessment areas. *Ameiurus melas* could negatively affect native ichthyofauna through direct predation and competition. This species is abundant in its native range; capable of securing and ingesting a wide range of food; gregarious; has a broad native range; high reproductive potential; longevity (10 years); highly adaptable to different environments; invasive in and outside its native range; a habitat generalist; tolerant of shade and poor quality waters

(Cucherousset et al., 2007). Given its characteristics, it can be considered potentially invasive for all the countries where it has established populations.

An indirect impact of *A. melas* on biodiversity can be through the generation of turbidity (e.g. Braig and Johnson, 2003 for the USA), which can reduce the feeding efficiency of visual-feeding native species (reviewed in Copp et al., 2016).

In the risk assessment area several North-American ictalurid fish species were introduced around 1900 for aquaculture purposes but also for stocking in impoverished European rivers. The latter proves the hardiness of these species and their ability to thrive is harsh conditions (Verreycken et al. 2010). Black bullhead is able to survive low oxygen concentrations for prolonged periods. It is a food generalist and has an omnivore diet. *Ameiurus* species are nocturnal zoophagophores, feeding on other aquatic species within the ecosystem. These species are predators of small fishes and larvae that have identical microhabitat requirements, such as aquatic invertebrates of which insect larvae are preferred. Ictalurid fish species feed on molluscs, fishes, algae, plant material and terrestrial invertebrates (Scott and Crossman, 1973; Brylinski and Chybowski, 2000; Leunda et al., 2008; Ruiz-Navarro et al., 2015). Black bullhead can even feed in turbid waters, by using its chin barbels (Scott and Crossman, 1973). *Ameiurus melas* predates on a wide variety of invertebrates, small vertebrates and fish eggs. Its parental care of eggs and young reduce mortality in the young and thus result in a higher survival. Moreover, it can erect its dorsal and pectoral spines as a defense against predators (Scott and Crossman, 1973).

In standing waters, this species can form dense populations (Keith et al., 2011). Moreover, ictalurid catfishes, including black bullhead, are potential vectors of non-native parasites (Scholz and Cappellaro, 1993; Uzunova and Zlatanova, 2007; Sheath et al., 2015).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Atlantic, Continental, Boreal & Mediterranean

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area

endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response:

- Croatia (Ćaleta et al., 2011)
- France (Cucherousset et al., 2006a)
- Germany (Nehring et al., 2015)
- Hungary (Koščo et al., 2010; Kováč, 2015)
- Italy (Amori et al., 1993; Novomeská et al., 2013)
- Poland (Nowak et al., 2010a, 2010b)
- Portugal (Garcia-de-Lomas et al., 2009; Miranda et al., 2010)
- Romania (Kováč, 2015)
- Slovakia (Koščo et al., 2010)
- Slovenia (Piria et al., 2016)
- Spain (Garcia-de-Lomas et al., 2009; Miranda et al., 2010)
- The Netherlands (NDFF and RAVON/ANEMOON, 2018)

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response: Ictalurid catfishes are not important in European aquaculture, but they have been or still are farmed in some countries, e.g. Italy (Bianco and Ketmaier, 2016; Sicuro et al., 2016).

The yearly European aquaculture production and value of *A. melas* in the early 2000s (mean for 2000–2004) is in 9th position (473.4 tons; 1,770,700 US$; value = 3.74 US$/kg) (Turchini and de Silva, 2008). *A. melas* has low benefits in sport fishing and very low benefits in the pet trade.

Production of *A. melas* from aquaculture in Europe (only Italy) excluding hatcheries and nurseries (from 2008 onwards) according to Eurostat (2018) varied between 43.2 t in 2013 to 245.75 t in 2010, with a mean yearly production of 148,2 t for the period 2010-2015.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided)”.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[2] and the provided key to pathways[3].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Q. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2–1.9

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[2] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[3] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Pathway name: N/A

- *Ameiurus melas* is already widespread in Europe, and currently there are no active introduction vectors, as the species is not known to be imported to the RA area from outside of the EU. So, the original vectors and pathways for the species introduction into Europe, i.e. fisheries (angling/sport purposes) and aquaculture, are no longer considered to be active. Unauthorised intentional and accidental releases are believed to be restricted to within and between members states and these are therefore assessed in the 'Entry' and 'Spread' sections.

Q. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and

storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely	**CONFIDENCE**	low

	unlikely moderately likely likely very likely		medium high

Response: N/A

End of pathway assessment, repeat Q. 1.3 to 1.7 as necessary using separate identifier.

Q. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied:

RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[4] and the provided key to pathways[5].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Q. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2–2.8

Pathway name:

a) RELEASE IN NATURE (Fishery in the wild)

b) ESCAPE FROM CONFINEMENT (“Aquaculture” and “Aquarium/garden pond species”)

a) RELEASE IN NATURE (Fishery in the wild)

Q. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional	**CONFIDENCE**	high

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[4] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[5] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Response: There is past evidence of intentional releases to the environment for the purposes of fish stocking and use in extensive (outdoor) aquaculture (Künstler, 1908; Wittenberg et al., 2006; Keith et al. 2011). Fish species valued by anglers are often reared in aquaculture facilities and then released into the wild to enhance local fish populations (i.e. stocking).

Q. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: The reputation of *A. melas* as a pest species by anglers (Cucherousset et al., 2006b) makes it less likely to be intentionally released in angling waters and less likely to be used in fish stockings, and its use in aquaculture also appears to have reduced dramatically except in certain localised areas.

Q. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	possible	**CONFIDENCE**	low

Response: Undetected entry into the environment could occur during the stocking of fish from sources where *A. melas* is present if adequate screening of the fish consignment is not implemented.

Q. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	**CONFIDENCE**	medium

Response: Fish stocking is normally undertaken during periods of the year that maximise potential survival, i.e. late winter/early spring, which coincides with the lead into the reproductive period.

Q. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: Similar to many species, *A. melas* can be spread accidentally or through intentional (but unauthorised) introductions (Nowak et al., 2010a, 2010b). However, *A. melas* is generally regarded as a nuisance species by anglers and therefore is less likely to be intentionally released in angling waters.

Q. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	possible	**CONFIDENCE**	medium

Response: In view of the continued aquaculture use of the species in some parts of the RA area, albeit relatively few, and the propensity of anglers and pet fish owners to release unwanted fish (e.g. Copp et al., 2005b), the likelihood of continued releases of this species into locations where it currently does not exist remains moderate.

End of pathway assessment, repeat Q. 2.2 to 2.7. as necessary using separate identifier.

b) ESCAPE FROM CONFINEMENT (“Aquaculture” and “Aquarium/garden pond species”).

Q. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	Medium

Response: There is evidence of accidental releases of other non-native fish species through the stocking of fish (e.g. Copp et al., 2010) and of aquatic plants (e.g. Copp et al., 2017), and the transportation and use of angling gear contaminated by fish eggs (Zięba et al., 2010). Examples of unintentional introduction via inter-basin (trans-watershed) water transfer schemes include the entry of non-native pikeperch *Sander lucioperca* (Wheeler, 1974), and the native spined loach *Cobitis taenia* from the River Great Ouse basin (East of England) via the Ely Ouse to Essex Transfer Scheme (see Copp & Wade 2006).

Q. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1–2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: Similar to many species, *A. melas* could be spread accidentally during fish stocking exercises (Copp et al., 2010; Nowak et al., 2010a, 2010b) or they could escape from aquaculture facilities during extreme hydrological events if the facilities are located on or near rivers (e.g. De Groot, 1985; Walker, 2004). However, the declining interest in the species for both angling and aquaculture suggests that large numbers are unlikely.

Q. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	possible	**CONFIDENCE**	low

Response: Unintentional release during fish stocking is less likely to be detected than accidental escape from aquaculture facilities, given that extreme hydrological events or loss of facility integrity will be noticed, and the loss of fish could, theoretically, be quantified. So, overall, the likelihood of detection is moderate.

Q. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	**CONFIDENCE**	medium

Response: Flooding events in Europe are normally during late winter/early spring, though in some cases during summer, which coincides with the lead into the reproductive period (spring) or the pre-autumn conditions that permit the fish the opportunity to escape and adapt to open waters and develop towards reproduction the following spring.

Q. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: Similar to many species, *A. melas* can be spread accidentally or through intentional (but unauthorised) introductions (Nowak et al., 2010a, 2010b). However, *A. melas* is generally regarded as a nuisance species by anglers and therefore is less likely to be intentionally released in angling waters.

Q. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	possible	**CONFIDENCE**	medium

Response: In view of the continued aquaculture use of the species in some parts of the EU (e.g. Italy; Eurostat, 2018), albeit relatively few, the likelihood of accidental escapes of the fish from aquaculture facilities, and for the accidental translocation of this species as a contaminate of authorised fish consignments, into novel locations remains moderate.

End of pathway assessment, repeat Q. 2.2 to 2.7. as necessary using separate identifier.

Q. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	possible	**CONFIDENCE**	medium

Response: Both intentional and unintentional releases of this species are possible at this time. However, confidence in this assessment is ‘medium’.

Q. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	possible	**CONFIDENCE**	medium

Response: Near-future climatic conditions are unlikely to modify the intentional use or the accidental release of this species, so scoring is the same as in Q2.8.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Q. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: This species is already established in several EU countries.

Q. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	widespread	**CONFIDENCE**	high

Response: In its native and introduced ranges, *A. melas* inhabits irrigation channels, lakes, ponds and reservoirs, which are principal habitats. Rivers and streams are secondary habitats (Scott and Crossman, 1973). *Ameiurus melas* is said to be most abundant in smaller water bodies, especially artificial and heavily managed ponds. It is considered a warm-water species (CABI, 2019; Copp et al., 2016; Leunda et al., 2008). There is an abundance of the species' preferred habitat types within the risk assessment area. *Ameiurus melas* can tolerate poor river conditions, and has a wide temperature tolerance, ranging between 8 and 30ºC (Baensch and Riehl, 1991; Cucherousset et al., 2007). Indeed, *A. melas* has the ability to tolerate, survive or adapt to a wide variety of environmental conditions. *Ameiurus melas* is a typical limnophilic species and one of the most tolerant fish species capable of resisting water pollution (Ribeiro et al., 2008; Nowak et al., 2010a). For example, Cucherousset et al. (2007) found *A. melas* to rank amongst the top two species in the Brière Marsh in terms of tolerance index, coefficient of water quality flexibility and temperature of upper avoidance. Increased eutrophication can benefit the growth of this species. The lack of native competitors and predators could lead to a further range expansion in the risk assessment area. The species' establishment following introduction has likely been facilitated by its life-history plasticity (Jarić et al., 2015; Copp et al., 2016; Jaćimović et al., 2019) and its generalist, omnivore diet with feeding aided, even in turbid waters, by its chin barbels (Scott and Crossman, 1973). All of these factors contribute to the *A. melas*'s high potential as a successful invader (Gante and Santos, 2002; Koščo et al., 2004; Dextrase and Mandrak, 2006; Copp et al., 2016), with the ability to occupy almost all the inland water surfaces in the risk assessment area. In particular, *A. melas* could especially become invasive in the southern parts with warmer waters (Scott and Crossman, 1973). Indeed, the numerous dams constructed for river regulation and as hydropower plants in Europe are an excellent opportunity for further expansion of its range (Cvijanović et al., 2005, 2008; Johnson et al., 2008).

Q. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	N/A	**CONFIDENCE**	high

Response: There is no evidence available to suggest that the species requires another taxon for any critical stage of its life cycle.

Q. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	high

Response: In a review of various studies, Copp et al. (2016) reported that the increasing trend in distribution and abundance of *A. melas* in some European countries has coincided with the decline of *A. nebulosus* (Nowak et al., 2010b). These contrasting patterns have led to suggestions that *A. melas* has been displacing *A. nebulosus*, but this is not true for Belgium where *A. melas* is not present and *A. nebulosus* is in decline (Verreycken et al., 2010). This was subsequently reviewed by Béres (2018): "The research findings confirm the hypotheses that the invasion of *A. melas* started and has not finished yet, and this species invading new habitats gradually replaces *A. nebulosus* not only in the natural waters in Hungary but even all over Europe (Harka 1997, Garcia-de-Lomas et al. 2009, Wilhelm 1998, Gante and Santo 2002, Luck et al. 2010, Popa et al. 2006, Nowak et al. 2010b, Kapusta et al. 2010, Movchan et al. 2014, Wilhelm et al. 1998)." By contrast, in the River Po, Italy, *A. melas* is reported to have declined in the 1990s following its introduction in the early 1900s (Castaldelli et al., 2013).

However, the two species have overlapping native distributions in North America (Fuller and Neilson (2017a, 2017b), so this pattern of *A. melas* replacement of *A. nebulosus* may simply be coincidental. However, further study is needed to determine whether or not this is an artefact or indicative of *A. melas* displacing *A. nebulosus*.

Q. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	high

Response: There is little information on how predators, parasites or pathogens could affect *A. melas*. Avian predators exist throughout the EU, but in Iberia, the only possible predatory fishes are non-native. In general, there does not appear to be any predators, parasites or pathogens in European water bodies, with a range of small native species likely to be the most impacted due to predation. By virtue of their strong pectoral and dorsal spines, which can lock into an erect position when threatened, adult *A. melas* are well protected from predation by all but the largest fish predators in their native range in Canada. Although present in juveniles, the spines are less robust, rendering juveniles more susceptible to predation by fishes with a wider range in size. Within its native range, predators include members of the families pike (*Esox* spp.) and pikeperch (*Sander* spp.) (Scott & Crossman, 1973; Hanchin et al., 2002), and there are representatives of both families in many parts of the risk assessment area.

Q. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	medium

Response: As with many fish species, it is virtually impossible to eradicate *A. melas* once established in a water course. However, in small, closed waters (e.g. small lakes or ponds), eradication of fish ,in general, may be possible by chemical means (e.g. rotenone) or by draining down of the water body (Britton et al., 2010b), including *A. melas* from an isolated pond in Essex, England (UK Environment Agency, 2014). Other known attempts to eradicate *A. melas* in the risk assessment area include intensive removals from the Brière Marsh, France which was only partly successful, probably because of the large area to be fished (Cucherousset et al., 2006a).

Q. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	unlikely	**CONFIDENCE**	low

Response: No evidence found to suggest management practices will facilitate the species' establishment, though in some countries inadequate screening of fish consignments (for stocking) could result in the accidental dispersal of *A. melas* (e.g. Copp et al., 2010).

Q. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	likely	**CONFIDENCE**	high

Response: Moore et al. (2010) classify *A. melas* as “high” about the “hardiness” criterion used as an indicator of the species’ ability to tolerate, survive, or adapt to a wide range of temperatures, pH, salt or freshwater aquatic environments, or the ability to survive out of water for extended periods of time. Indeed, *A. melas* has considerable tolerance of water pollution, turbidity, low oxygen concentration, elevated temperatures and a range of pH values (Cucherousset et al., 2007; Novomeská et al., 2013). The species biological traits appear to facilitate the ability of *A. melas* to recover from population crashes (Jaćimović et al., 2019) and unsuccessful eradication attempts (Marchetti et al., 2004). As a result of this tolerance and their bottom habit, *A. melas* is most difficult to eradicate both physically and chemically, the species being less sensitive to the piscicide ‘rotenone’ than some other species (Ling, 2002). That said, successful eradication of *A. melas* from a small pond in Essex, England, the only know extant population of *A. melas* in the UK, has been reported (UK Environment Agency, 2014). An unsuccessful attempt in France to eradicate *A. melas* from the Brière Marsh, France, by intensive removals involved the use of traps and electrofishing equipment (Cucherousset et al., 2006a).

Q. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very likely	**CONFIDENCE**	medium

Response: This species is already established within the RA area. *A. melas* become sexually mature between ages 1–3 years (Copp et al., 2016) (with maximum reported age of 10 years (Froese & Pauly, 2019)) and are relatively fecund, producing between 2 000 and 3 800 eggs during each spawning period. Males guard the nest for up to 10 days after hatching (Etnier and Starnes, 1993), and then the young-of-the-year juveniles form a dense ball-shaped shoal that follows the female around until the older juveniles begin to disperse. The feeding behaviour *of A. melas* is omnivorous/generalist/opportunistic, and the species demonstrates life-history plasticity (Scott and Crossman, 1973; Ribeiro et al., 2008; Jarić et al., 2015; Copp et al., 2016; Jaćimović et al., 2019).

Additionally, *A. melas* is resistant to domestic and industrial pollution (Scott and Crossman, 1973) and can survive in a range of temperatures (0–25^{o}C), with an upper lethal temperature of 23–35°C (Scott and Crossman, 1973). The species is also said to withstand low dissolved oxygen levels (0.3 mg/L) (CABI, 2019). In Moore et al. (2010), all *Ameiurus* species, except *A. serracanthus*, are said to present a moderate population growth, according to the criterion "resilience", which indicates the rate of population doubling as an indicator of the rate of potential population growth.

Q. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	likely	**CONFIDENCE**	high

Response: The species' tolerance of a vast array of water quality variables enhances its ability to adapt to, and live in, a range of freshwater habitats, including those threatened with drought (Cucherousset et al., 2007). This is apparent in the species' establishment in various global locations outside its native range, including Europe (Copp et al., 2016) and western North America (Scott and Crossman, 1973; Forbes and Flook, 1985). The species' feeding behaviour is omnivorous/generalist/opportunistic, and it demonstrates considerable life-history plasticity (Scott and Crossman, 1973; Ribeiro et al., 2008; Jarić et al., 2015; Copp et al., 2016; Jaćimović et al., 2019).

Q. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	possible	**CONFIDENCE**	low

Response: No evidence was found to suggest that low genetic diversity would reduce this species' chances of establishment. A genetic study of North American populations (native range) found that *A. melas* is "relatively stable over time or the population is comprised of more geographically structured sub-populations" (Padhi, 2010). The reported expansion of *A. melas* in Central Europe (Béres, 2018) would suggest that there are no genetic constraints on the species in Europe.

Q. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	possible	**CONFIDENCE**	medium

Response: As stated here above, *A. melas* inhabits lakes, ponds, reservoirs, rivers and streams, brackish waters, estuaries and irrigation channels, and it is able to tolerate, survive, or adapt to a wide range of temperatures, pH, salt or freshwater aquatic environments (Scott and Crossman, 1973). As such, failure to establish is unlikely, but if establishment is not achieved, then persistent as a casual is very likely, though a casual fish is not likely to persist beyond 10 years (max. lifespan is about 10 years (Froese & Pauly, 2019)).

Q. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very likely	**CONFIDENCE**	high

Response: Both the native and EU ranges of this species encompass five climate type zones (Peel et al., 2007), with four of these shared by the native and EU ranges (Cfa, Dfa, Dfb, Dfc), as such establishment in the risk assessment area, even in other parts where it is not yet established, is very likely under current climatic conditions.

Q. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.
With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30–50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9–2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very likely	**CONFIDENCE**	high

Response: With climate change affecting the water temperature, an increase in water temperature is likely to facilitate this species’ (Britton et al., 2010a) establishment in more areas. Britton et al. (2010a) ran a comparison of mean Climatch scores between 2009 and 2050 for *A. melas* in the UK. *Ameiurus melas* has an increased climate match with the source region in 2050 when compared with 2009. This species is likely to benefit from climate warming in England and Wales, this prediction was then tested using water temperature modeling. One can expect that similar benefit is true for regions between 50° and 55° N as modeled by Britton et al. (2010a). As such, it is likely to establish in more areas where previously the water temperature would be too low to reproduce. This would facilitate establishment in countries with a current colder climate such as UK (Britton et al., 2010a) and Poland, where the species was already reported within the last decade (Nowak et al., 2010a). The increase in temperature would allow *A. melas* to spread and establish more widely into all biogeographic regions except probably the Alpine.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic “spread” via release or escape (“jump-dispersal”), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Q. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	moderate	**CONFIDENCE**	medium

Response: Within its native range in North America, most natural dispersal of *A. melas* has occurred at local levels (Fuller et al., 1999). In European waters, the dispersal mechanism of *A. melas* is not clear, but it is likely to be associated with accidental and illegal introductions (Nowak et al., 2010a, 2010b), combined by natural spread between neighboring countries via natural and human-made water courses (Panov et al., 2009). Despite being established in several European countries for over a century, the natural dispersal of *A. melas* has been relatively slow. Dense populations have formed in standing waters only, with movements of adult *A. melas* tending to be localised (Bouvet et al., 1982, 1985). After hatching, the young of both *A. melas* and its close congener, *A. nebulosus*, form dense ball-shaped shoals that follows the female around for approximately a month prior to local dispersal. Therefore, this species is less likely to spread rapidly than some other species.

Nonetheless, *A. melas* is now the most widespread North American ictalurid catfish in Europe (Pedicillo, 2008), being widely dispersed in some countries, e.g. Italy (Bianco, 1998), France (Keith et al., 2011) and Portugal (Almaça, 1995), but localised in others, such as Spain (Doadrio et al., 1991), Germany (Arnold, 1990), and formerly in England (Lever, 1977; Copp et al., 2016) where it is now possibly extirpated (UK Environment Agency, 2014).

Q. 4.2. How important is the expected spread of this organism within the risk assessment area

by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	moderate	**CONFIDENCE**	high

Response: As reported here above, *A. melas* can be spread accidentally or through intentional (but unauthorised) introductions (Nowak et al., 2010a, 2010b), but because it is generally regarded as a nuisance species by anglers, it is less likely to be released intentionally by anglers. This would be a reversal of past practices in Poland, where intentional introductions of its close congener (*A. nebulosus*) continued up to about the year 2000 (Witkowski, 2002; Kapusta et al., 2010) leading to the introduction of *A. melas* presumably as a contaminant species (Kapusta et al., 2010; Nowak et al., 2010a, 2010b). The intentional stocking of *A. melas* for recreational fishing purposes has decreased in recent years. In Czech Republic, quite recently, evidence was obtained on unintentional introduction of *A. melas* with carp stocking from Croatia to the fishponds in the Třeboň district in 2003 (Koščo et al., 2004; Lusk et al., 2010). The expansion was human helped in some cases, for example it was imported to Hungary from Italy in 1980 (Harka, 1997).

Just outside the EU, in Serbia, there’s poor control of the stocking procedure. Apart from the small carp, some amount of *A. melas*, pumpkinseed *Lepomis gibbosus* and topmouth gudgeon *Pseudorasbora parva* are always found in the stocking material. Thus, many Serbian waters are still being unintentionally stocked with non-native fish (Lenhardt et al., 2010), including locations where *A. melas* has established (Jaćimović et al., 2019). In Ukraine, *A. melas* was probably introduced together with the commercial fisheries introduction of *A. nebulosus*, where it has become invasive locally but is said to be spreading rapidly (Kvach and Kutsokon, 2010).

Q. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.

- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway names: a) CORRIDOR - Interconnected waterways/basins; and b) RELEASE IN NATURE - Other intentional release.

a) CORRIDOR - Interconnected waterways/basins

Q. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: Secondary, natural dispersal on an organism following its release is most likely to be an unintentional consequence of the entry, both the intentional release and the unintentional escape, of organisms into a new drainage basin (assessed in the ‘Probability of Entry’ section here above).

Q. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	possible	**CONFIDENCE**	medium

Response: Following the intentional release or the unintentional escape of organisms into a new drainage basin (assessed in the ‘Probability of Entry’ section here above), the number of individuals involved in secondary dispersal within the new drainage basin would depend on the numerical size of that basin’s source population and on the connectivity between the point source and the remainder of the drainage basin. However, it is possible that there would be sufficient numbers dispersing over the course of the year, given the likelihood of floods/spates during certain seasons, which increase connectivity (e.g. Copp, 1989; Amoros and Bornette, 2002).

Q. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and

storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Natural dispersal does not involve storage, but survival during natural spread (i.e. ‘transport’) along water courses and canals is very likely, with subsequent reproduction possible. Indeed, the reproduction of some fish species is triggered and facilitated by inundation of the flood plain (e.g. northern pike *Esox lucius*). For *A. melas*, little is known of their migrations, except for movements within and between floodplain water bodies in France (Bouvet et al., 1985; Cucherousset et al., 2007). The distribution of *A. melas* in a partially-abandoned side-channel (Lône des Pêcheurs) of the Upper River Rhône was observed by Bouvet et al. (1982) to be relatively uniform along its 1.6 km extent. Marked *A. melas* in that side-channel were reported to bury themselves in the sediments during winter (Bouvet et al., 1985), and once emerged post-winter, the species were abundant until March, but disappeared in the spring, returning each year in the autumn at the same location where initially captured, thus demonstrating site fidelity. Within the side channel, displacements of the marked *A. melas* ranged from 0 to 900 m to the channel’s upstream extent, and up to 640 m in a downstream direction. The presence of young-of-the-year *A. melas* in this same side channel during summer (Copp, 1989) suggests that not all adults migrate out, or that adults from elsewhere migrate into such off-river habitats to spawn. This migratory behaviour in *A. melas* is, not surprisingly, similar to that of its close congener, *A. nebulosus* (Sakaris et al., 2005).

Q. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Unless there is a specific monitoring programme and rapid-response protocol that targets pest fish species, it is very likely the organism would survive existing management practices because their dispersal along water ways will not be detected. There is a multitude of bibliographic sources that demonstrate the difficulty of detecting rare fish species in running waters.

Q. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: Unless there is a species-specific sampling programme that involves conventional and environmental DNA detection methods, the species’ spread along water ways will be detected only by anglers perhaps and/or incidental encounters during routine monitoring.

Q. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	likely	**CONFIDENCE**	medium

Response: Most water ways involve proximity to some form of flood plain that contains still waters, which are the preferred habitat of *A. melas* (Scott and Crossman, 1973) and their young-of-the-year (Copp, 1989), and most EU water courses are subjected to floods and spates that result, even in regulated systems, in the overflow of the water course into the adjacent flood plain.

Q. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	slowly	**CONFIDENCE**	medium

Response: In European waters, natural spread of *A. melas* could be within and between countries via water courses (Panov et al., 2009; Nowak et al., 2010a, 2010b). However, as described here above, *A. melas* is a relatively sedentary species (Bouvet et al., 1982, 1985), which suggests relatively-low natural dispersal.

b) RELEASE IN NATURE - Other intentional release

Q. 4.3b. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	**CONFIDENCE**	high

Response: Secondary, human-assisted dispersal of an organism, following its entry into a previously-unoccupied drainage basin, whether by intentional release and via unintentional escape (assessed in the ‘Probability of Entry’ section here above), can result in the movement of the species within the new drainage basin by intentional human translocation (e.g. Copp et al., 2005b).

Q. 4.4b. How likely is it that a number of individuals sufficient to originate a viable population

will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	moderately likely	**CONFIDENCE**	low

Response: This will depend upon how many fish were released (or escaped) into the ‘point of origin’, so confidence is low.

Q. 4.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Human-assisted storage and transport is normally undertaken with the intention of maximum survival of the organism, so as to achieve the intended purpose at the point of new release. So, it is very likely that the organism will survive the relatively short translocation within the same drainage basin for release to a previously-uninhabited part of that drainage basin. Reproduction is highly unlikely during such short transport and/or storage.

Q. 4.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very likely	**CONFIDENCE**	medium

Response: With regard to releases for the purpose of stocking the fish into a new location within the same drainage basin, whether authorised or not, management of the fish stocks by the person(s) undertaking the release of fish can be assumed to be with the intent of the species’ survival. In the case of an unauthorised release, existing management practices of the government authorities are unlikely to affect the survival of the translocated fish except if they disperse out of the stocked (intended) location into adjacent waters that are subject to control of government agencies. As such, survival of existing management practices is highly likely.

Q. 4.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	unlikely	**CONFIDENCE**	high

Response: Unless there is a species-specific sampling programme that involves conventional and environmental DNA detection methods, the species' spread along water ways will be detected only by anglers perhaps and/or incidental encounters during routine monitoring. In the case of unauthorised releases within the same drainage basin, these are likely to be clandestine and therefore unlikely to be detected.

Q. 4.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very likely	**CONFIDENCE**	high

Response: In the case of intended release to a new part of the same drainage basin, this is assumed to be into suitable habitat, so as to achieve the purpose of the stocking, whether authorised or not.

Q. 4.9b. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	slow	**CONFIDENCE**	medium

Response: Unlike natural dispersal of *A. melas*, which is relatively slow, translocations by humans would normally be at least moderate if the species is of interest. However, *A. melas* is generally considered to be a nuisance/pest (Nowak et al., 2010a, 2010b), so translocation of this species, whether authorised or not, is likely to be slow.

End of pathway assessment, repeat Q. 4.3 to 4.9. as necessary using separate identifiers.

Q. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in

relation to these pathways of spread?

RESPONSE	difficult	**CONFIDENCE**	high

Response: It is well known that containment and eradication of fish, once in a water course, is difficult, if not impossible (e.g. Tyus and Saunders, 2000). Basically, the likelihood of containing and extirpating a fish species from a water course is inversely related to the size (width, depth, water discharge) of the water course. Whereas, containment and potential eradication is possible in smaller, enclosed waters (Britton et al., 2010b).

Q. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	slow	**CONFIDENCE**	medium

Response: As described here above, *A. melas* is a relatively sedentary species, which suggests relatively slow natural spread.

Q. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	slow	**CONFIDENCE**	medium

Response: Relatively little is known of the dispersal potential of *A. melas*, but in its close congener, *A. nebulosus* in its native North American range, telemetry studies have demonstrated a preference for warmer waters (Kelso, 1974; Richards and Ibara, 1978; Crawshaw et al., 1982; Sakaris et al., 2005), which could suggest that an increase in mobility may be expected under warmer climate conditions. Both of these *Ameiurus* species appear to share a sedentary existence (e.g. Bouvet et al., 1982, 1985; Sakaris et al., 2005; Millard et al., 2009), suggesting that any such increased mobility of *A. melas* is likely to be modest. Most water ways involve proximity to some form of flood plain that contains still waters, the preferred habitat of *A. melas* (Scott and Crossman, 1973; Copp, 1989), and the incidence (frequency and intensity) of extreme hydrological variations is projected to increase in many EU water courses under future climate conditions. This would result, even in regulated systems, in the overflow of the water course into the adjacent flood plain, thus enhancing the dispersal of *A. melas*, though not as rapidly as species of greater, natural migratory inclination.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Q. A.7)

Biodiversity and ecosystem impacts

Q. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	major	**CONFIDENCE**	medium

Comment: A review of non-native species in British Columbia (Voller and McNay, 2007) reported *A. melas* to be an omnivorous bottom forager that feeds heavily on molluscs, so the species can pose a threat to endangered mollusc species. Other studies have reported a clear impact, that *A. melas* can extirpate a *Gasterosteus* population in two years (Cannings and Ptolemy, 1998), with predation of *Gasterosteus* eggs (Backhouse, 2000). In their translocated North American range, introduced *A. melas* prey on endangered humpback chub *Gila cypha* in the Little Colorado River, which is believed may significantly affect the native species by depleting numbers and reducing recruitment (Marsh and Douglas, 1997). Introduced *A. melas* is believed to be at least partially responsible for the decline of the Chiricahua leopard frog *Rana chiricahuensis* in southeastern Arizona (Fuller and Neilson, 2017). Hughes and Herlihy (2012) conclude that piscivorous alien fishes, which included *A. melas*, are associated with reduced population sizes of native prey species, at least during the summer low-flow period, and are potential threats to prey species persistence.

A major concern with *A. melas* is its association with degraded or impacted ecosystems, which are considered more susceptible to invasion (Moyle, 1986), and the increased turbidity created by *A.*

melas in mesocosm experiments (Ohio, USA) can exert impacts on ecosystem function (Braig and Johnson, 2003).

The species' close congener, *A. nebulosus* is known to have extirpated the *Gasterosteus* species pair from a lake in British Columbia, Canada (Hatfield, 2001). In the Pacific Northwest, there are several lakes where the only native fish species is *Gasterosteus aculeatus*, which is present in distinct limnetic and littoral forms.

Q. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	major	**CONFIDENCE**	medium

Comment: Savini et al. (2010), in a review of the impacts caused by the most important 25 aquatic alien species intentionally introduced in European waters, recorded ten references of potential impacts by *A. melas*: bioaccumulation (storage and magnification toxic substances in tissues), community dominance (species causing quantitative changes in community structure in becoming the dominant species), competition (for food or for space with native species) and predation (predatory activity on native species). However, the references for these citations are not provided either in Savini et al. (2010) nor in the contract report (Occhipinti Ambrogi et al., 2008) from which that article was derived. Gozlan (2010) reported that *A. melas* was the fish species introduced to Spain that posed the greatest potential for ecological impact, however, without citing the sources of supporting evidence.

In the species' introduced European range, there has in fact been little study of the species' impacts (Copp et al., 2016), and most simply examined the species' diet and that of native species, information upon which inferences of threats to native species have been made. For example, a coincidental disappearance or decline in native (and Iberian endemic) species, with an increase in the number of alien species, including *A. melas*, was reported for the Doñana wetland, southern Spain (Moreno-Valcárcel et al., 2012).

One of the rare studies to demonstrate direct predation by *A. melas* was in a small pond in England where roach *Rutilus rutilus*, a very common species through most of the EU, was found to represent 30% of the diet (Ruiz-Navarro et al., 2015). That study was undertaken just prior to the eradication of *A. melas* from that pond (UK Environment Agency, 2014). However, in areas of the EU characterised by elevated endemism, predation on endemics poses a considerable threat to biodiversity. The most prominent study has been of *A. melas* piscivory in three Iberian river systems: in one river, the main fish prey were native (endemic) species whereas in the two other rivers *A. melas* piscivory mainly involved alien invasive fishes (Leunda et al., 2008), see more details further down.

In France, experimental studies have found that the predation efficiency of age-1 native northern pike *Esox lucius* was reduced in the presence of age-1 *A. melas* due to behavioural interference (Kreutzenberger et al., 2008). Whether or not this interference, within the relatively small confines of the 200 L tanks, is replicated in nature remains to be seen – an important issue because laboratory-demonstrated interactions are not necessarily observed between the co-occurring species in open waters (see Kakareko et al., 2016).

Another, indirect impact of *A. melas* on biodiversity can be through the generation of turbidity (e.g. Braig and Johnson, 2003), which can reduce the feeding efficiency of visual-feeding native species (reviewed in Copp et al., 2016). In order to assess environmental and economic impacts of alien and invasive fish species in Europe using the generic impact scoring system, Van der Veer and Nentwig (2015) calculated the impact points obtained by the generic impact scoring system in six environmental impact categories for *A. melas*. (herbivory, predation, competition, transmission of diseases, hybridization and ecosystem alteration). Comparing with the mean score for the 40 alien established fish species, five of the scores for environmental impact (except Hybridization) were greater in the case of *A. melas*.

In the Slovak part of the middle Danube (Slovakia), the virtual disappearance of small benthic native species (e.g. European bullhead *Cottus gobio*, white-finned gudgeon *Gobio albipinnatus*, stone loach *Barbatula barbatula*) from the local fish communities coincided with invasive non-native fishes, which included *A. melas* (Černý, 2006; Novomeská et al., 2016). In Hungary, *A. melas* is listed as coming to dominate the fish community but no impact are identified (Bódis et al., 2012).

In Spain and Portugal, Leunda et al. (2008) showed that *A. melas* are preying on native fish species such as *B. graellsii*, *P. miegii* and *G. lozanoi*. Even if only fish bony remains (e.g. scales, opercula, cleithra and pharyngeal arches) were identified in *A. melas* stomachs, egg predation could not be excluded. Probably, egg predation was not detected because of rapid digestion. Due to the generalist and opportunistic feeding habits of this species, Leunda et al. (2008) analysed data from Spain and Portugal indicating impacts on a wide range of potential prey species as well as impacts through competition. In this study, *A. melas* consumed plant material, terrestrial prey and co-occurring fish species (native or exotic), taking the most abundant and available prey. Therefore, this species might be reducing the amount of available prey for native predators.

Leunda et al. (2008) found that the diet composition of *A. melas* is similar to the diet described for some co-occurring Iberian native species. Taking into account the voracity and aggressive behavior of *A. melas,* the diet similarity might lead to an unfavourable competition for the same food resources, subsequently, displacing native fishes to suboptimal food resources. And in a lagoon in the Spanish province of Zamora, *A. melas* is considered the cause of decline of the common parsley frog *Pelodytes punctatus* and the Iberian painted frog *Discoglossus galganoi* (MAPAMA, 2013).

Q. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	major	**CONFIDENCE**	medium

Comment: This species is able to establish across a wide range of climatic zones, so the predicted warmer conditions for virtually all of the EU is unlikely to modify the likely magnitude of impacts by *A. melas* in the future.

Q. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	major	**CONFIDENCE**	high

Comment: There is evidence from the risk assessment area of potentially negative impacts such as predation on native species by *A. melas* (see comments in response to Q. 5.2), however evidence of competition (for food and/or space) requires further study, given that competition can be difficult to demonstrate. Changes in water transparency, due to increased turbidity (Braig and Johnson, 2003), could affect all the ecosystems where this species is present.

Under the Water Framework Directive (WFD), any decline in native species, and/or an increase in non-native species, can affect ecological status, however in their study, Hermoso et al. (2010) did not include *A. melas* in their calculations of Index of Community Integrity for the River Guadiana (Spain) because the species' prevalence was below 5%.

A few examples of the presence of *A. melas* in sites of nature conservation value include:
Spain, where *A. melas* is present (but no information on impacts is provided) in the:
• National Park Tablas de Daimiel (Dirección General de Política Forestal y Espacios Naturales. Junta de Comunidades de Castilla La Mancha, 2015).
• Doñana Natural Area (Moreno-Valcárcel et al., 2012).
• Biosphere Reserve and Regional Park "Cuenca Alta del Manzanares" near the city of Madrid (Pino-del-Carpio et al., 2010).

France, where *A. melas* is included in the list of fish species recorded (but no information on impacts is provided) on the Natura 2000 site of the Lower Valley Doubs - Doubs and Clagu (Muséum National d'Histoire Naturelle, 2016).

Q. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk

assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	moderate	**CONFIDENCE**	low

Comment: If current climate carries on getting warmer, this suggests that this species could spread more rapidly than the current 'slow' spread, and this species could have a greater adverse impact on native species and aquatic ecosystems that are the subject of conservation interest and legislative protection.

Ecosystem Services impacts

Q. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	minimal	**CONFIDENCE**	low

Comment: Although *A. melas* is mentioned in papers that discuss non-native species impacts on ecosystem services (e.g. Gozlan, 2010), no evidence of ecosystem services impact is presented. However, in water bodies used by anglers, their perception of the angling value may be reduced by the species' presence (unpublished statements from discussions with anglers). For example, *A. melas* can cause a painful sting if pectoral spines puncture human flesh due to the small amounts of venom at the ends of spine, which can cause pain for up to a week (Rose, 2006; Etnier and Starnes, 1993). However, scientific studies of the impacts on ecosystem services (e.g. decline in use of water bodies due to invasive fish presence) are lacking. Ictalurid catfishes can also pose a public health risk, if eaten, due to their accumulation of contaminates when inhabiting polluted waters (review by Savini et al., 2010; Department of Environmental Conservation. New York State, 2017).

Q. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Q. 5.6.

RESPONSE	minor	**CONFIDENCE**	low

Comment: As mentioned above, painful wounds can be inflicted by the sharp spines in the fins of *A. melas* if they are not handled carefully, and *A. melas* have been found to contain elevated levels of contaminants, which poses a risk in cases where this species is taken from contaminated waters and eaten.

Q. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Q. 5.6.

RESPONSE	N/A	**CONFIDENCE**	**-**

Comment: No published evidence has been found that would allow to answer this question.

Economic impacts

Q. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minor	**CONFIDENCE**	low

Comment: The economic impact assessment by Van der Veer G. and Nentwig (2015) indicated ‘0’ impacts for *A. melas*, which is likely to have been based on the absence of information rather than hard evidence, given that no studies are known to have been undertaken on the economic losses associated with *A. melas*. In certain cases of wild establishment, *A. melas* introductions have the potential to hinder local commercial and sport fisheries through competition with target species (CABI, 2019). There is also potential that *A. melas* can have a negative economic impact on communities as this fish can be a “nuisance” species taking lines/bait intended for other species. Anglers not targeting this species might therefore move on to *A. melas* free waters, taking not only the money from recreational fishing but tourism (food, accommodation and transportation), all of which may provide economic opportunities locally (Godard, 2015).

Q. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minor	**CONFIDENCE**	low

Comments: Anglers are in general annoyed by this species, which takes their baits and is difficult, due to its poisonous spines, to remove from their fishing lines, and the species can increase turbidity levels in some cases (as mentioned above). This suggests the potential for a reduction in the perceived social and economic value of waters infested by *A. melas*. The scarcity of published evidence on this suggests that impacts are sufficiently minimal as not to warrant study. That said, a study for Great Britain and Ireland (Gallardo and Aldridge, 2013) included *A. melas* in the list of 12 aquatic species potentially causing greatest ecological and economic harm. However, there was only one confirmed population of *A. melas* in Great Britain and Ireland – it was located in an isolated, private-owned field and located a long distance from any connecting water course, and that population was eradicated in 2014 (UK Environment Agency, 2014). As such, more information is needed in order to estimate the potential economic costs of *A. melas* in the EU.

Q. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Q. 5.10.

RESPONSE	minor	**CONFIDENCE**	low

Comments: Currently, there is no technical or scientific data upon which to estimate such costs. In the event that *A. melas* benefits from future climate conditions and expands its EU range, then one may assume that there would be a reduction in the perceived social and economic value of waters infested by the species.

Q. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	minor	**CONFIDENCE**	medium

Comments:

It can be assumed that, although widely spread in the risk assessment area, overall no systematic effort has been undertaken to manage the species across the RA area.

The economic costs of eradication of *A. melas* could be relatively modest or very high, depending upon the extent of the species' spread, the size of the water bodies it invades, etc. The cost of the operation to remove *A. melas* from the small pond in Essex (UK Environment Agency, 2014) was ≈£5000–£10000 (≈€5400–€10900), including personnel costs (Animal and Plant Health Agency, personal comm.), however all of the angling club's fish were lost due to the rotenone treatment.

Similar range of costs are reported by other invasive fish eradications in the U.K., e.g. for topmouth gudgeon *Pseudorasbora parva*, it was found that the costs of eradication increase with increasing larger waterbody size (e.g. Britton et al., 2010, 2011), but on average £20K GBP per hectare (≈ €22k/ha) (Britton et al., 2008).

Another example is the cost of eradicating northern snakehead *Channa argus* from a small pond in Crofton, Maryland (U.S.A.), which was estimated to be $110k USD (≈ €100k). This included personnel time for planning meetings, field application of the piscicide, and disposal of the dead fish (Courtenay and Williams, 2004). In 2010 alone, the US federal government committed $78.5 million in investments to prevent the introduction of Asian carp to the Great Lakes, where they would threaten Great Lakes fisheries and could negatively impact remaining populations of endangered or threatened aquatic species (U.S. Fish and Wildlife Service, 2012).

Q. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Q. 5.12.

RESPONSE	minor	**CONFIDENCE**	low

Comments: The costs identified in the comments to Q. 5.12 would be expected to increase should the species spread more widely, as is suggested in the 'Spread'.

Social and human health impacts

Q. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal	**CONFIDENCE**	medium

Comments: *Ameiurus melas* can cause a painful sting if pectoral spines puncture human flesh. *Ameiurus melas* contain small amounts of venom at the ends of spine which can cause pain for up to a week. (Rose, 2006; Etnier and Starnes, 1993). Additionally, *A. melas* could pose a public health risk if consumed due to its accumulation of contaminates when inhabiting polluted waters (Savini et al., 2010; Department of Environmental Conservation. New York State, 2017).

Q. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	minimal	**CONFIDENCE**	low

Comments: As mentioned above, painful wounds can be inflicted by the sharp spines in the fins of *A. melas* if they are not handled carefully, and *A. melas* have been found to contain elevated levels of contaminants, which poses a risk in cases where this species is taken from contaminated waters and eaten.

Other impacts

Q. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	major	**CONFIDENCE**	high

Comments: *A. melas* is a susceptible species to host *Aphanomyces invadans* related to the Epizootic ulcerative syndrome. In the EU Regulation 2018/1882[6], *A. melas* is listed as vector of Viral Haemorrhagic Septicaemia (VHS) and Infectious Haematopoietic Necrosis (IHN). The species also hosts *Edwardsiella ictaluri* in liver and spleen kidney. This parasite is related to *Enteric septicaemia* of catfish and *Edwardsiellosis* (Buller, 2014). *Ameiurus melas* also hosts *Flavobacterium columnare*, which is related to the Columnaris disease (Buller, 2014), and it is highly susceptible to two ranaviruses: European Catfish Virus (ECV) and Epizootic Haematopoietic Necrosis Virus (EHNV) (Gobbo et al., 2010). Ranaviruses pose a potential threat to fishes and amphibians.

The *A. melas* population in England has also been shown to host *Ancyrocephalus pricei* population (Sheath et al., 2015). In Italy, *A. melas* has been attributed to the introduction of the exotic cestode *Corallobothrium parafimbriatum*, though further spread of the cestode with its fish host to other countries has not been reported. *Acanthocephalus anguillae*, adopted by *A. melas*, is the common parasite of native fishes (about 40 species) in Slovakia (Košuthová et al., 2009).

Q. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal	**CONFIDENCE**	medium

Comments: None have been encountered in the literature search.

Q. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	major	**CONFIDENCE**	low

Comments: It is not well known how predators, parasites or pathogens could affect *A. melas* and any information available indicates there are no other organisms that would control it naturally: At least in

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[6] https://eur-lex.europa.eu/legal-content/en/TXT/PDF/?uri=CELEX:32018R1882&from=EN

Spain, the only possible piscivorous fishes will also be non-native, there are such predators of *A. melas* in both its native and introduced European ranges, i.e. members of the pike family (*Esox* spp.) and pike perches (*Sander* spp.). However, some piscivorous fishes are unable to predate ictalurid catfishes, including both *A. melas* and *A. nebulosus*, due to their sharp, strong dorsal and pectoral spines that may lock into an erect position when predated upon (Mandrak, 2009). Although present in juveniles, the spines are less robust making juveniles more susceptible to predation by fishes with a wider range in size. These spines, combined with the species' nocturnal feeding regime, make *A. melas* an uncommon prey item for most fish species. However, some piscivorous birds, such as cormorants and herons, as well as some turtle species, will occasionally consume the young and small adults of ictalurid catfishes (CABI, 2019).

Q. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	moderate	**CONFIDENCE**	low

In the species' introduced European range, there has in fact been little study of the species' impacts (Copp et al., 2016). The scarcity of evidence of impacts in the risk assessment area, mainly due to a lack of such studies, makes it difficult to assess the species current impacts. In view of the species' relatively limited current, localised distribution, the overall impacts in the RA area are likely to be moderate, being minimal-to-minor in some areas and perhaps moderate-to-major in specific areas.

Q. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	moderate	**CONFIDENCE**	low

Although the potential enhancement of establishment potential under conditions of climate warming is likely, the scarcity of evidence of impacts in the risk assessment area, mainly due to a lack of such studies, makes it difficult to assess the species current and future impacts. The overall impacts in the RA area in the future are likely to be moderate, being minimal-to-minor in some areas and perhaps moderate-to-major in specific areas.

RISK SUMMARIES

	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction*	very unlikely	medium	*A. melas* is already present in several EU countries due to previous introduction vectors (aquaculture, ornamental use and sports fishing), but there are no active introduction vectors. New introductions from its native regions (N. America) therefore seem very unlikely, consequently also new entries in the EU are very unlikely. Transport between and within member states remains possible (see Spread section).
Summarise Entry*	possible	medium	Unauthorised introductions of *A. melas* by anglers have occurred within and between EU countries in the past and is likely to continue, though perhaps less frequently due to the declining interest for aquaculture and the increasing anglers' view of the species as a pest.
Summarise Establishment*	very likely	high	*Ameiurus melas* is established in several EU countries, but there is some evidence of populations declining. *Ameiurus melas* can inhabit a wide range of freshwater ecosystem, and therefore could potentially adapt easily to the climatic conditions in some countries where it currently does not exist, if allowed to be translocated. The species is tolerant of poor water quality, including contaminants, and a wide range of water temperatures. The lack of native competitors and predators in some locations could lead to a further range expansion in Europe, though in other locations native predators (pikes, pikeperches) are present. The degree of invasiveness of *A. melas* is facilitated by its plasticity in life-history traits, its

			parental care, its elevated tolerances to poor water quality conditions, and its generalist/opportunistic feeding behaviour.
Summarise Spread*	slow	medium	*A. melas* has been established in several European countries for over a century now and natural dispersal seems to be slow. The spread of *A. melas* through human-assisted intentional (and accidental) introductions seems to be rather slow as *A. melas* is often regarded as a nuisance species by anglers and therefore increasingly less likely to be intentionally released in angling waters.
Summarise Impact*	moderate	medium	*Ameiurus melas* may affect the native fauna in various ways, including: 1) predation on native species, especially threatened/protected species; 2) resource exploitation and/or behavioural interference, which deprives, or reduces the access of, native species of food; 3) increased turbidity, which can modify the feeding efficiency of visual predators; and 4) physical injury (from the spines of *A. melas*) to native predators (e.g. snakes, fish) that attempt to predate *A. melas*. There are some reports of impacts in Europe and elsewhere, which highlight the need to consider occurrences of *A. melas* in sites of nature conservation interest, e.g. national parks and nature reserves. Hybridisation with native species is extremely unlikely, if not impossible, given that the Family Ictaluridae is not native to the risk assessment area. So, hybridisation is possible only with other non-native ictalurid catfishes present in the RA area, e.g. *A. nebulosus* and channel catfish *Ictalurus punctatus*.

			Ameiurus melas is a susceptible species to host bacteria, fungi and other organisms. It is highly susceptible to two ranavirus. Ranaviruses pose a potential threat to fishes and amphibians. Although there are no detailed studies of economic losses due to this species, in some cases, *A. melas* introductions have had the potential to hinder local commercial and sport fisheries through interference with the commercial/sport species (CABI, 2019). Published studies that report on the economic costs associated with managing this species derive from North America and from the U.K., providing a means to estimate costs per unit area of infested water body whereby eradication feasibility is greater in still water sites than in running waters, and feasibility decreasing in both types of water with increasing size *Ameiurus melas* can cause a painful sting if pectoral spines puncture human flesh, which affects anglers' perceptions of a water body, thus lowering the social and economic value of infested water bodies.
Conclusion of the risk assessment (overall risk)	moderate	medium	The species' distribution includes several EU countries but populations are localised and there is one report of *A. melas* declining (River Po, Italy). Introduction is unlikely due to vectors and pathways having mostly ceased to operate, but intentional and accidental releases of *A. melas* into open waters and translocations from existing populations continue to pose a moderate risk. This potential for entry to open waters is probably the main means of dispersal of the species, which is known to be

			relatively sedentary, so natural spread is slow. The fact that the species has established in various EU countries evidences its relatively high risk of establishment. Potential impacts include increased turbidity, especially in smaller water bodies and potential decreases in the ecosystem services (mainly angling), with some concern expressed over *A. melas* presence in national parks and nature reserves (especially in Iberia), though studies of economic loss produced by *A. melas* are lacking. Other potential impacts include the transmission of fish diseases to some fish species native to most of the EU (e.g. European catfish *Silurus glanis*).

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:
Yes if recorded, established or invasive
– if not recorded, established or invasive
? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	Yes	Yes	Yes	Yes	-
Belgium	Yes	-	Yes	Yes	-
Bulgaria	Yes	-	Yes	Yes	-
Croatia	Yes	Yes	Yes	Yes	Yes
Cyprus	-	-	Yes	Yes	-
Czech Republic	Yes	Yes	Yes	Yes	-
Denmark	-	-	Yes	Yes	-
Estonia	-	-	Yes	Yes	-
Finland	-	-	Yes	Yes	-
France	Yes	Yes	Yes	Yes	Yes
Germany	Yes	Yes	Yes	Yes	Yes
Greece	-	-	Yes	Yes	-
Hungary	Yes	Yes	Yes	Yes	Yes
Ireland	-	-	Yes	Yes	-
Italy	Yes	Yes	Yes	Yes	Yes
Latvia	-	-	Yes	Yes	-
Lithuania	-	-	Yes	Yes	-
Luxembourg	-	-	Yes	Yes	-
Malta	-	-	Yes	Yes	-

Netherlands	Yes	Yes	Yes	Yes	Yes
Poland	Yes	Yes	Yes	Yes	Yes
Portugal	Yes	Yes	Yes	Yes	Yes
Romania	Yes	Yes	Yes	Yes	Yes
Slovakia	Yes	Yes	Yes	Yes	Yes
Slovenia	Yes	Yes	Yes	Yes	Yes
Spain	Yes	Yes	Yes	Yes	Yes
Sweden	-	-	Yes	Yes	-
United Kingdom	Yes	Yes	Yes	Yes	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	-	-	-
Atlantic	Yes	-	Yes	Yes	-
Black Sea	-	-	Yes	Yes	-
Boreal	Yes	Yes	Yes	Yes	Yes
Continental	-	-	Yes	Yes	-
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian	Yes	Yes	Yes	Yes	-
Steppic	-	-	Yes	Yes	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea	-	-	-	-	-
Black Sea	-	-	-	-	-
North-east Atlantic Ocean	-	-	-	-	-
Bay of Biscay and the Iberian Coast	-	-	-	-	-
Celtic Sea	-	-	-	-	-
Greater North Sea	-	-	-	-	-
Mediterranean Sea	-	-	-	-	-
Adriatic Sea	-	-	-	-	-
Aegean-Levantine Sea	-	-	-	-	-
Ionian Sea and the Central Mediterranean Sea	-	-	-	-	-
Western Mediterranean Sea	-	-	-	-	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[7]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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[7] Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition,*

			predations, spread of disease etc.)
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[8]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*

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[8] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*

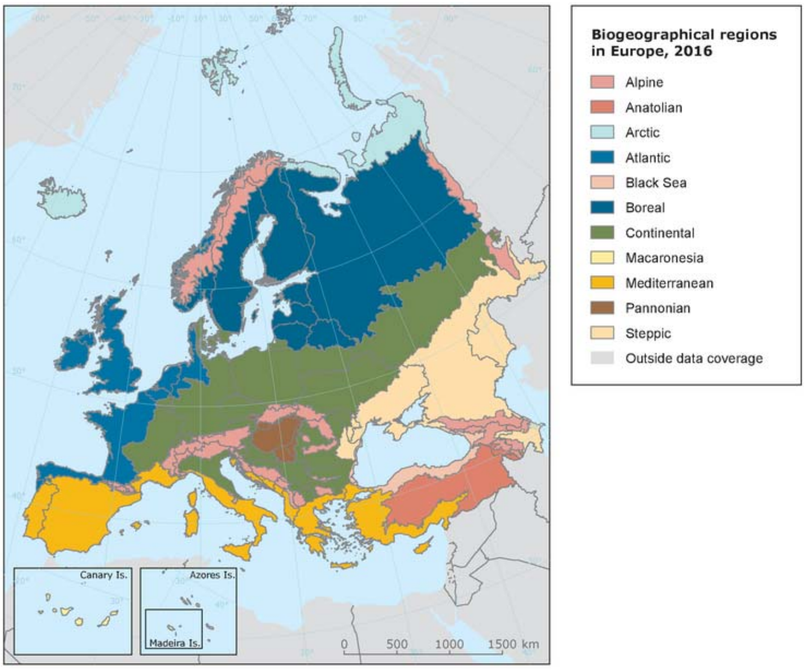
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

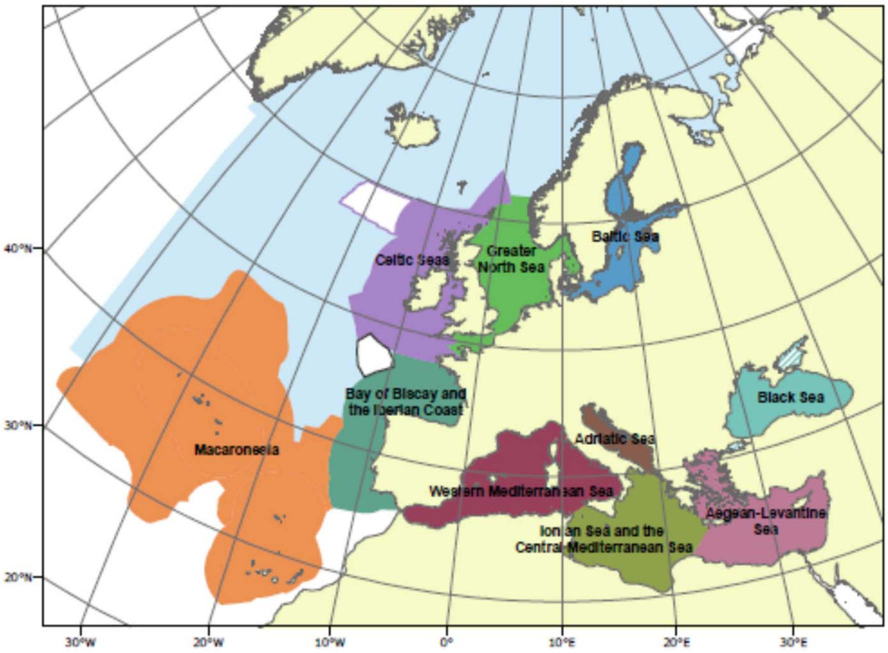
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	*Ameiurus melas* (Rafinesque, 1820)
Species (common name)	**Black bullhead**
Author(s)	**H. Verreycken, L.R. Aislabie, G.H. Copp**
Date Completed	**23 October 2019**
Reviewer	**Ján Koščo**

Summary [1]

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

As *A. melas* is already widespread in the EU and there are no relevant pathways of introduction and entry in the RA area. Previous pathways for introduction into Europe are not considered relevant anymore. Unauthorised introduction by anglers is estimated to be the main origin for new within and between Members States introductions and spread. The adoption and enforcement of appropriate legislation and codes of best practice could reduce the likelihood of introduction. The most cost-effective way of preventing the intentional introductions is to raise public awareness of the problems associated with the establishment of *A. melas.* It will educate people on the impacts of IAS and if people become more educated they are less likely to release them in the environment and more likely to report IAS when they see them.

Early detection of the species in newly infested sites is very hard due to the areas this species inhabits and their benthic, hidden life. The use of eDNA is a new promising tool here. Additional reporting from recreational fishermen of new findings of this fish would benefit the targeted monitoring for *A. melas*. Although fish surveys (e.g. for the WFD) would be a continuous way of early detection it is a very expensive measure and it is not guaranteed that the species will be detected.

Rapid eradication of the species is dependent on where and at what stage it is found. The potential to eradicate or control *A. melas* populations depends on dispersal location and the level of establishment. In small enclosed water bodies piscicides (rotenone) could be effective in eradicating populations but one should be attentive and respect the legal constraints on the use of piscicides in the EU.

Mechanical removal using fyke nets, electrofishing and other fishing gear may be successful to manage ictalurid catfish populations in small confined areas. Targeted angling on this species can also be a part of the removal exercise of this species.

There is very limited literature in relation to costs associated with prevention, eradication and management programmes for *A. melas*.

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)**[3]	**Level of confidence**[4]
Methods to achieve prevention [5]	**Managing the pathways:** *Ameiurus melas* was introduced in Europe in the late 1800s and is already widespread here. At present, there are no active vectors of introduction but entry to the environment may continue to occur (unauthorised) via historical vectors, i.e. angling/sport purposes (within and between members states), aquarium release, and aquaculture, though all are in decline. The adoption and enforcement of appropriate legislation and codes of best practice could reduce the likelihood of introduction to the EU as well as further (secondary) spread through translocation of the species to new waters (entry to the environment).	Enforcement of appropriate legislation and codes of best practice would be an effective means of limiting the risk of intentional spread of *A. melas* by anglers between and within member states. There is no information available about the costs and the equipment or infrastructure that may be required to implement this measure, but it is widely accepted that prevention is more cost effective than management of already introduced species. These measures would need to include the provision and training of administration and staff to enforce the regulations.	Medium
	Increasing public awareness,	Campaigns to educate people and increase awareness on	Medium

	including education and training to reduce the risk of intentional (and un-intentional) introduction and spread: It is important to raise awareness about the possible consequences of introducing and spreading *A. melas* to non-infested sites in the EU. It will also diminish the chance of new introductions after eradication/management of an invasive species. The production of targeted publicity and identification material is needed.	IAS are an effective way to curb (il)legal introductions, especially those targeted at specific sectors e.g. anglers. Public awareness campaigns, however, do need to be maintained so they do not drop out of the collective consciousness, but are also renewed periodically to avoid fatigue. Ideally the development of awareness raising campaigns and educational materials needs to be done for each member state, guided by scientific expertise and co-ordinated by an "education committee" or a similar initiative. Resources required, and associated costs, are dependent upon the activities and materials developed, but may include media campaigns, websites, marketing materials, or outreach training and education schemes (Roy et al., 2018). Costs of campaigns to increase awareness are estimated to be low to medium (€50–200K/year) on an EU scale. These campaigns typically cover more than one non-native (invasive) species thus spreading the costs per species.	
Methods to achieve eradication [6]	**Effective surveillance and reporting:** *Ameiurus melas* is a well-known species although confusion with its close congener, brown bullhead *Ameiurus nebulosus*, can be a problem. Effective eradication is most likely to be achieved when new invasions are quickly reported. Encouraging rapid reporting of new incursions	Trawl nets, fyke nets, traps, and electrofishing can be used to detect and monitor for non-native fishes in the RA area, even if not always effective at low density (Britton et al., 2011) in which case and environmental DNA approaches can be used (e.g. Dougherty et al., 2016; Davison et al., 2017, 2019), including in large lakes (Larson et al., 2017). Environmental DNA based monitoring could be considered also for the entire RA area. Citizen science could be promoted to monitor the possible introduction and spread of the species. If dedicated monitoring for *A. melas* is not possible, then	Medium

	increases the likely success of rapid response before the species can become established. Post-eradication detection can also be undertaken to determine whether or not an eradication action has been successful. A simple and clear identification sheet could be drafted and distributed to different stakeholders (e.g. anglers, fishery managers) to increase the probability of an early detection and rapid response. Early detection can be enhanced through monitoring that involves the use of environmental DNA analysis of water samples – this is discussed further here below under "Reducing risks of further dispersal".	monitoring for this species can be incorporated in already running monitoring programmes e.g. for the Water Framework Directive. Although the above mentioned tools may be effective in early detection, eradication of *A. melas* after first find can only be effective when the detected infestation is low, with the potential feasibility and effectiveness of the eradication dependent on the size and type of infested water, still waters being easier than water courses, and feasibility decreases (and costs increase) with increasing size of the water needing eradication action. In large riverine systems, which are a typical habitat of *A. melas*, eradication may be impossible (see also below). The costs of dedicated surveillance and monitoring and subsequent removal of invasive fish are estimated to be medium (€200K–1M for five years) for the RA area.	
	Depletion and/or drain down of small standing waters: *A. melas* is most commonly associated with stillwater environments, but it does occur in water courses.	Drainage can be efficient and cost-effective, but is only feasible in some types of water bodies (e.g. fish ponds, small river reservoirs) and can be very destructive when rare or valuable fish species and other aquatic biota are negatively affected (e.g. Britton et al., 2010; Davison et al., 2019). Therefore, preferably, most fishes are mechanically removed prior to draining. The following methods may be suitable for depletion sampling and removal of fishes in the EU: electrofishing, seine nets, minnow traps, and fyke nets.	Medium

		The likelihood of successful eradication, however, is low except in relatively small closed waters. Similar drain-down (or de-water) eradications in the UK of topmouth gudgeon *Pseudorasbora parva* have been in ponds estimated from the articles to have been 0.7 and 2.95 ha (Britton et al., 2008, 2010). Although this type of measure can be successful in eradicating *A. melas* populations, it is more suitable to be used as a management method. Cost are likely to be medium to high (>€50k/ha)	
	Use of piscicide (chemical removal): a piscicide can be used to kill newly-detected populations in smaller areas such as ponds, drainable larger water bodies (e.g. reservoirs), or small water courses.	*A. melas* can be killed by rotenone or other piscicides. However, it would be difficult (if not impossible) to make an effective eradication in large rivers. Use of rotenone was already successfully used for eradicating the invasive topmouth gudgeon *Pseudorasbora parva* in the UK (Britton et al., 2008, 2010) as well as *A. melas* from a small, isolated 2 ha pond in Essex, England (UK Environment Agency, 2014). The largest water body in these rotenone eradications of *P. parva* was a small lake of 2.95 ha (Britton et al., 2008). Use of rotenone for the control of invasive fishes is widespread in the U.S.A. (Ling, 2002, U.S. Fish & Wildlife Service - Environmental Conservation Online System, 2019), and has also been successfully applied in South Africa to eradicate the invasive smallmouth bass *Micropterus dolomieu* in a 4 km reach of the Rondegat River (Weyl et al., 2013, 2014). There may be legal constraints as e.g. Rotenone was withdrawn from use in the European Union in 2007 (Schapira, 2010), but is still used in the U.K. (Britton et al.,	High

		2008, 2010; UK Environment Agency, 2014). The potential to eradicate or control *A. melas* populations depends on dispersal location and the level of establishment. If broadly dispersed in large lakes or river systems, eradication or control would likely be impossible. The costs for of applying rotenone are high and were calculated for six ponds and lakes in the UK (for the eradication of topmouth gudgeon). The costs as ranging from £6,600 (€7,500) for a small pond (0.23 ha) to £61,000 (€70,000) for a small lake (2.95 ha) (Britton et al., 2010). Eradication costs per surface area have been estimated in the U.K. as on average £2 GBP/m^2 (i.e. £20K GBP/ha, (≈ €22k/ha) (Britton et al., 2008). In the USA, however, rotenone treatments of large areas to remove (but not completely eradicate) common carps and ictalurid catfishes were reported to be less expensive (U$25,000 for 31 ha (≈ €730/ha), U$33,000 for 60 ha (≈€499/ha) and U$40,000 for 492 ha (≈€74/ha) (U.S. Fish & Wildlife Service - Environmental Conservation Online System, 2019). In Hawai, the control of tilapia with rotenone (CFT Legumine) was estimated to cost U$5000 for 81 ha (€56/ha)(only product was counted, not personnel and equipment)(Tavares, 2009).	
Methods to achieve management [7]	**Mechanical removal:** Mechanical removal of *A. melas* can be done by fyke and seine netting, and electrofishing. Protocols for removal are well developed for a wide variety of fishes (West et	Mechanical removal may be the only way to reduce abundance of an invasive fish where chemical piscicides cannot be applied. Fyke and/or hoop nets have been effective in removing large numbers of *Ameiurus* catfishes and are a standard method to collect this species in many jurisdictions, e.g. New Zealand (Barnes & Hicks, 2003),	High

	al., 2007). Electrofishing is preferred because it has the least amount of by-catch and damage to native fish populations (Mueller, 2005). Angling can also be locally effective in removing large numbers of some invasive fishes (Savior, 2016).	North America (Miranda & Boxrucker, 2009; Pope et al., 2009) and Europe (Louette & Declerck, 2006; Cucherousset et al., 2006). A combination of fyke or hoop nets and electrofishing are considered good forms of mechanical removal for ictalurid catfishes (Prott et al., 2006; Miranda & Boxrucker, 2009). Double fyke nets, consisting of two conically shaped fyke nets (mesh size of 8 mm) of which the mouth openings are connected with a vertically hanging net (length, 11 m; height, 0.9 m), have been used effectively in Belgium (Louette & Declerck, 2006). These methods are not normally used on their own for eradication because they are not 100% effective (see Davison et al., 2017) and may be selective for size/age classes. Combined use of traps and/or electrofishing with chemical piscicides may, however, result in successful eradication. Repeated removal attempts will result in higher efficiency. Angling and increased fishing effort by amateurs could also be part of the overfishing effort (Savior, 2016). Finally, the possibility of combing mechanical removal with drastic habitat alteration may also help or increase the synergistic pressures on an isolated population of invasive fishes such as these. *Ameiurus melas* is difficult and costly to control (CABI, 2015). Data on detailed specifics and costs are generally lacking and are case specific e.g. Britton et al. (2010) estimated the cost for a near eradication of topmouth gudgeon (15 specimens left after five biomanipulation exercises) in a small (0.3 ha) and shallow (<1.5 m) lake to be	

		£2,100. Investing in electrofishing equipment and fyke nets are needed. Additionally, gill nets and seine nets can be used. Prices of electrofishing gear are estimated to range from €750 to €4000 and more; fyke nets are between €500 and €1500, while gill nets are cheaper, but different mesh sizes need to be applied and high mortalities to non-target fishes may occur. The biggest costs are for operating these fishing gears and are an important extra cost to consider, as they are very labour intensive.	
	The **above methods** described to support eradication can also be used to manage existing *A. melas* populations.	See above	See above
	Reducing risks of further dispersal	Dedicated monitoring (e.g. electrofishing, fyke nets, trawl nets but also eDNA) of water courses and water bodies is necessary to detect the presence of *A. melas* and to ensure that these waters are not recolonised by the species after eradication. In parallel, to prevent further spread and new introductions, a prohibition on the keeping and release should be enforced. Also, stringent procedures should be put in place to check within-EU consignments of fish intended for angling. Depending on the area that has to be monitored, management costs can be from medium to very high (from <€5k to > €1M).	Medium
	Further research	Additional research on *A. melas* is needed within the EU to understand better the risks posed by the species (e.g. Copp et al., 2016) and also the extent of the species distribution,	Medium

		which remains less well known than some other non-native species in Europe, e.g. pumpkinseed *Lepomis gibbosus* (Copp & Fox, 2007).	

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: *Ameiurus nebulosus* (Lesueur, 1819)
Common name: brown bullhead

Authors of the assessment:

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Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: *Sebastian Kozic, Department of Ecology and Vertebrate Zoology, University of Lodz, Łódź, Poland*

Peer review 2: *Phillip J. Haubrock, Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt, Germany*

Date of completion: 23 October 2019

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: *Ameiurus nebulosus* belongs to the genus *Ameiurus* (Rafinesque, 1820) and is part of Siluriformes (catfishes), Ictaluridae (Gill, 1861) (North American freshwater catfishes) (Froese & Pauly, 2019):

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Ictaluridae

Ictaluridae comprises eight genera (one extinct) and 67 species—51 living (12 with fossil records) and 16 extinct (Arce-H. et al., 2016). The monophyly of living Ictaluridae is well supported by molecular data using parsimony and model-based methods. The analysis found further support for the monophyly of the genus *Ameiurus*, which is represented by 16 species of which nine are fossils (Arce-H. et al., 2016).

From the Integrated Taxonomic Information System (www.itis.org) *Ameiurus* species include:

- *Ameiurus brunneus* Jordan, 1877 – snail bullhead
- *Ameiurus catus* (Linnaeus, 1758) – white catfish, white bullhead
- *Ameiurus melas* (Rafinesque, 1820) – black bullhead
- *Ameiurus natalis* (Lesueur, 1819) – yellow bullhead
- *Ameiurus nebulosus* (Lesueur, 1819) – brown bullhead

- *Ameiurus platycephalus* (Girard, 1859) – flat bullhead
- *Ameiurus serracanthus* (Yerger and Relyea, 1968) – spotted bullhead

Synonyms (non-valid) for *A. nebulosus* are *Pimelodus nebulosus*, *Ictalurus nebulosus* and *Ictalurus nebulosus nebulosus*. Common name for *A. nebulosus* is brown bullhead but also used are brown catfish, common bullhead or horned pout (Froese & Pauly, 2019)

Ictalurid catfish species (also referred to as bullheads) have an adipose fin between their dorsal and tail. Ictalurid catfishes have a rounded tail, which will help distinguishing them from small channel catfish *Ictalurus punctatus*, which has a forked tail. Ictalurid catfishes have no scales, their bodies are covered with taste buds, and will be very slippery to handle. Finally, ictalurid catfishes have a single, sharp spine in the dorsal and pectoral fins. Like other members of the family, ictalurid catfishes also have barbels (‘whiskers’) under their chin that help them locate food (Scott and Crossman, 1973).

In the risk assessment area, currently only brown *A. nebulosus* and black bullhead *A. melas* are established. Other species (white and yellow bullhead) were only recorded very occasionally. There are a number of reports of the introduction of *A. natalis* (yellow bullhead) into Italy (Welcomme, 1988; Holčík, 1991). However, there is no reliable evidence for this (Godard, 2015). Confirmed presence exists for *Ameiurus catus* (white catfish) only in the UK (Britton and Davies, 2006; Zięba et al., 2010).

Ameiurus nebulosus (brown bullhead) is known to hybridise naturally with their close congeners *A. melas* and *A. natalis* (Hunnicutt et al., 2005).

The species in the genus are sometimes very difficult to distinguish from each other, especially *A. melas* and *A. nebulosus* (Wheeler, 1978).

One of the main distinguishing features that distinguish *A. melas* and *A. nebulosus* is that the *A. melas* has a weak serration on the trailing edge of the pectoral spines; whereas for *A. nebulosus*, the pectoral spine edge comprises regular saw-like barbs. The colour pattern also varies with *A. melas* being mainly dark, whereas *A. nebulosus* is usually mottled, but may be solid also (Allen and Godard, 2015). An important feature to distinguish *A. melas* and *A. nebulosus* is the colouration of the caudal and anal fin membrane (Decru and Snoeks, 2011): *A. melas* always has a black-and-white radiation on the caudal and anal fins, whereas *A. nebulosus* clearly does not have this. *Ameiurus melas* has lightly coloured fin rays with the tissue between the fin rays always dark, which causes this black-and-white radiation. For *A. nebulosus* the entire fins are rather light in colour.

Confusion between species could be possible, so identification of other species in the genus as *A. melas* or *A. nebulosus* cannot be ignored (Lenhardt et al., 2011).

The known common names of the brown bullhead in other European languages than English are the following:

barbotte brune (Québecois), Zwergwels, Brauner Katzenwels (German), kanalnyi somik (Russian, Ukrainian), bruine Amerikaanse dwergmeerval (Dutch), sumik karłowaty (Polish), brun dvärgmal (Swedish), dvergmalle (Norwegian), poisson chat and barbotte brune (French), piikkimonni (Finnish), sumcek krpatý (Slovak), sumecek americký (Czech), somn American and bici-cu-coarne (Romanian), brun dværgmalle (Danish) (Global Invasive Species Database, 2019).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: There are two spcies of the genus *Ameiurus* in the risk assessment area, the black and the brown bullhead (Wheeler, 1978). There is a high degree of morphological similarity between *A. nebulosus* and *A. melas*. Differences have been mentioned in the previous question.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: *A. nebulosus* has been ranked, using the Fish Invasiveness Screening Kit (FISK: Copp et al., 2009), in several European countries/regions as representing a 'medium' or 'high' risk of being (or becoming) invasive in those risk assessment areas:

Ameiurus nebulosus was classified as 'high' risk for England & Wales (Copp et al., 2009).

Puntila et al. (2013) concluded that the risk of invasion for south of Finland is 'medium–high'.

Simonović et al. (2013) categorise the species as 'high' risk to become invasive for the Balkans Region.

Ameiurus nebulosus is categorised also as 'high' risk of being invasive for Croatia and Slovenia (Piria et al., 2016).

The species is categorised as 'moderately-high' risk of being invasive in the drainage basin of Lake Balaton, Hungary (Ferincz et al., 2016).

The species is categorised as 'high' risk of being invasive in Greece (Perdikaris et al., 2016).

According to Nehring et al. (2010), it is invasive in Germany and according to Wiesner et al. (2010) it is potentially invasive in Austria. It also listed on the German "Black List" (Schwarze Liste) https://neobiota.bfn.de/fileadmin/MDB/documents/service/skript285.pdf.

In Australia, *A. nebulosus* was identified as posing a medium-high risk of becoming invasive using FISK (Vilizzi and Copp, 2013), and as a potentially high-risk, noxious species using a rapid risk assessment approach that was developed in Australia to assess the potential impact of ornamental fishes on the environment and other species if released into the wild. That report assessed the potential risks posed by 447 ornamental fish species on the national grey list (Moore et al., 2010). The Department of Fisheries of the Government of Western Australia (2013) included this species in the State's Noxious Fish List.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: Native to North America: Atlantic and Gulf Slope drainages in the Canadian provinces of Nova Scotia and New Brunswick to Mobile Bay, Alabama, USA, and from the St Lawrence River/Great Lakes, Hudson Bay and Mississippi River basins from Quebec west to Saskatchewan in Canada and south to Louisiana, U.S.A. (Froese and Pauly, 2019).

This species may have been originally absent from all or part of the Gulf Coast, west of the Apalachicola and east of the Mississippi River. This speculation is based on the very spotted distribution of the species both in panhandle Florida and Alabama although it appears to be largely confined to reservoirs in Alabama. In its native range in peninsular Florida, it is found primarily in larger water bodies. Whereas, on the Atlantic Slope in Florida, this species is found in both streams and sloughs (Fuller and Neilson, 2017b).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: It has been introduced outside of its native range in North America to other areas of North America, Asia and Pacific islands (i.e. New Zealand, Hawaii). Also, in Chile, Iran and Turkey (Iriarte et al., 2005; Salvador Vilariño, 2015; Froese and Pauly, 2019).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic.

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea.

Marine subregions:

- Greater North Sea, including the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a): Atlantic, Boreal, Mediterranean, Pannonian.

Response (6b): Atlantic, Boreal, Mediterranean, Pannonian.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on:

- The applied timeframe (e.g. 2050/2070);
- The applied scenario (e.g. RCP 4.5);
- What aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods).

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, then original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9–2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Following Climatch (Peel et al. 2007) all biogeographic regions, except the alpine, of the risk assessment area have at current climate more or less suitable climate for establishment of *A. nebulosus*.

Atlantic Region

Black Sea Region

Boreal Region

Continental region

Mediterranean region

Pannonian Region

Steppic Region

Response (7b): Britton et al. (2010a) ran a comparison of mean Climatch scores between 2009 and 2050 for *A. melas*, a close congener of and from the same native region as *A. nebulous*, in the UK. *Ameiurus melas* has an increased climate match with the source region in 2050 when compared with 2009. This species is likely to benefit from climate warming in England and Wales, this prediction was then tested using water temperature modelling. One can expect that similar benefit is true for regions between 50° and 55° N as modeled by Britton et al. (2010a).

Atlantic Region

Black Sea Region

Boreal Region

Continental biogeografical region

Mediterranean biogeografical region

Pannonian Region

Steppic Region

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom (see Movchan et al., 2014.)

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): There are records of *A. nebulosus* in 17 EU Member States:

- Austria (Wiesner et al., 2010; Kováč, 2015) – first record unknown
- Belgium (Verreycken et al., 2007) – first record *ca.* 1882
- Bulgaria (Olenin et al., 2008, Global Invasive Species Database, 2019) – first record *ca.* 1975
- Croatia (Piria et al., 2016); – first record *ca.* 1920
- Czech Republic (Lusk et al., 2010) – first record *ca.* 1890
- Denmark (Mandrak, 2009; Rutkayová et al., 2013) – first record unknown
- Finland (Urho and Lehtonen, 2008) – first record *ca.* 1922
- Germany (Wolter et al., 2000; Nehring et al., 2010) – first record *ca.* 1885
- Greece (Barbieri et al., 2015) – first record *ca.* 2012
- Hungary (Mandrak, 2009; Rutkayová et al., 2013) first record *ca.* 1902
- Italy (Wheeler, 1978) – first record *ca.* 1900
- Poland (Kapusta et al., 2010; Witkowsk et al., 2002) – first record *ca.* 1885
- Romania (Petrescu and Mag, 2006; Kováč, 2015) – first record *ca.* 1910
- Slovakia, (Kováč, 2015; Secretariat of NOBANIS, 2012) – first record *ca.* 1925
- Slovenia (Povž, 2017; Piria, 2016) – first record *ca.* 1975
- The Netherlands (Olenin et al., 2008; NDFF and RAVON/ANEMOON, 2018) – first record *ca.* 1900
- UK (Olenin et al., 2008; Mandrak, 2009; Rutkayová et al., 2013; Wheeler, 1978) – first record *ca.* 1885

Response (8b): There are established populations in 16 EU Member States. Most introductions ended in established populations but establishment dates are almost never published. In general, establishment date is not so much different from date of first record (see 8a):

- Austria (Wiesner et al., 2010; Kováč, 2015)

- Belgium (Verreycken et al., 2007, 2010)
- Bulgaria (Uzunova and Zlatanova, 2007)
- Croatia (Treer et al., 2008)
- Czech Republic (Holčík, 1972; Lusk et al., 2010)
- Denmark (Carl and Møller, 2012)
- Finland (Urho and Lehtonen 2008)
- Germany (Wolter et al., 2000; Nehring et al., 2010),
- Greece (Barbieri et al., 2015)
- Hungary (Guti et al. 1991; Juhász et al., 2013; Guti & Pekarik, 2016)
- Italy (Wheeler, 1978)
- Poland (Grabowska et al., 2010; Rechulicz and Płaska, 2018)
- Romania (Antonescu, 1938; Petrescu and Mag, 2006; Kováč, 2015)
- Slovakia (Koščo et al., 2010)
- Slovenia (Povž, M., 2017)
- The Netherlands (Leuven and Oyen, 1987; NDFF & RAVON/ANEMOON, 2018)

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on:

- The applied timeframe (e.g. 2050/2070);
- The applied scenario (e.g. RCP 4.5);
- What aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods).

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9–2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): This species could establish in the following EU Member States, which currently are not known to have established populations:

- Cyprus
- Estonia
- France (Cucherousset et al., 2006b). Note that Bruslé & Quignard (2001) reported that *A. nebulosus* was introduced to France at the same time as *A. melas* but failed to establish.
- Ireland
- Latvia
- Lithuania
- Luxembourg
- Malta
- Portugal
- Spain (Clavero, 2011)
- Sweden
- UK (Copp et al., 2009)

Response (9b): Same as 9a, see question 7b for establishment under climate change.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:

Collier et al. (2017) assessesd the species-specific Invasion Risk Impact scores (the product of predicted invasion risk and species impact) of non-native freshwater fishes in New Zeeland and highlighted Eurasian perch (*Perca fluviatilis*) and the brown bullhead (*A. nebulosus*), as the species most likely to spread and cause ecological harm in lakes in New Zeeland. *A. nebulosus* is known to have extirpated two *Gasterosteus* species from a lake in British Columbia, Canada (Hatfield, 2001). Iriarte et al. (2005) reported that *A. nebulosus* is of the exotic species that may be considered truly invasive in Chile , on account of their naturalization in the wild and their spread over the country. However, although many publications consider *A. nebulosus* as potentially invasive no other papers could be found that actually provide evidence of adverse impacts on biodiversity outside the risk assessment area.

In the risk assessment area several North-American ictalurid fish species were introduced around 1900 for aquaculture purposes but also for stocking in impoverished European rivers. The latter proves the hardiness of these species and their ability to thrive is harsh conditions (Verreycken et al. 2010). Brown bullhead is able to survive low oxygen concentrations for prolonged periods. It is a food generalist and has an omnivore diet. *Ameiurus* species are nocturnal zoophagophores, feeding on other aquatic species within the ecosystem. These species are predators of small fishes and larvae that have identical microhabitat requirements, such as aquatic invertebrates of which insect larvae are preferred. Ictalurid fish species feed on molluscs, fishes, algae, plant material and terrestrial invertebrates (Scott and Crossman, 1973; Brylinski and Chybowski, 2000; Leunda et al., 2008; Ruiz-Navarro et al., 2015). Brown bullhead can even feed in turbid waters, by using its chin barbels (Scott and Crossman, 1973). *Ameiurus nebulosus* predates on a wide variety of invertebrates, small vertebrates and fish eggs. Its parental care of eggs and young reduce mortality in the young and thus result in a higher survival. Moreover, it can erect its dorsal and pectoral spines as a defense against predators (Scott and Crossman, 1973).

In standing waters, this species can form dense populations (Keith & Allardi, 1998). Louette and Declerck (2006) recorded the density and made biomass estimates for brown bullhead populations in several ponds in Flanders (Belgium); density ranged between 393 and 2022 individuals per ha and a biomass between 7.2 and 50.5 kg/ha.

Its benthic feeding habit can increase turbidity and lead to altered productivity and nutrient cycling (Scott and Crossman, 1973).

Nearly all risk assessments (see A3) rank *A. nebulosus* as ‘medium’ or ‘high’ risk of being (or becoming) invasive in the member states of the risk assessment areas. *Ameiurus nebulosus* could negatively affect native ichthyofauna through direct predation and competition. Furthermore, the diet of the large size classes of brown bullhead has been found to consist almost exclusively of juvenile fishes. This indicates that brown bullhead populations potentially affect recruitment of indigenous fish populations(Louette and Declerck, 2006). Given its characteristics, it can be considered potentially invasive for all the countries where it has established populations.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic.

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea.

Marine subregions:

Greater North Sea, including the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Boreal, Continental & Mediterranean

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Response: *A. nebulosus* shows signs of invasiveness in four EU Member States:

- Finland (Koli, 1990; Urho et al., 1995)
- Germany (Nehring et al., 2010)
- Poland (Paduszek, 1996; Grabowska et al., 2010; Rechulicz and Płaska, 2018)
- Romania (Petrescu and Mag, 2006)

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, then qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response: *Ameiurus* species are not amongst the species that historically dominated European aquaculture and they are not an alternative to salmonid or cyprind farming. At the same time, in recent years, in the complex panorama of European inland aquaculture, the appearance of new exotic species for culture purposes should be considered with concern (Turchini et al., 2008). Bianco and Ketmaier (2016) also reported that *A. nebulosus* is used in aquaculture in Italy but no indication of volume or value is provided. *A. nebulosus* nor *A. melas* are in aquaculture in The Netherlands (Van der Valk et al., 2018).

Economic benefits from *A. nebulosus* aquaculture occur primarily within Chile, China, Bulgaria and Belarus (Welcomme, 1988; Tan and Tong, 1989; Reshetnikov et al., 1997; Mikhov, 2000), although the magnitude of these benefits remains uncertain. Introduced populations of *A. nebulosus* to Europe and some Pacific islands originally provided social benefits as sportfish (Welcomme, 1988), but their current social value as sportfish within their introduced range is low and has poor economic value. *Ameiurus nebulosus* is popular in some areas as a gamefish. The species is reportedly a good eating fish, especially when smoked (Mandrak, 2009).

Production from aquaculture in Europe (Croatia and Romania) has been reported by Eurostat (2018) to have decreased slightly from 5.8 tonnes live weight in 2008 to 4.03 in 2012.

A. nebulosus is considered as a good bait fish for other larger game fish like *Silurus glanis* (in Italy) and *Pylodictis olivaris* (in the USA)(P. Haubrock, pers. comm.)

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided)”.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[2] and the provided key to pathways[3].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Q. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9

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[2] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[3] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Vector and Pathway names: N/A

Ameiurus nebulosus is already widespread in Europe (Piria et al., 2018), and currently there are no active introduction vectors, as the species is not known to be imported into the EU. So, the original vectors and pathways for the species introduction into Europe, i.e. fisheries (angling/sport purposes) and aquaculture, are no longer considered active. Unauthorised intentional and accidental releases are believed to be restricted to within and between members states and these are therefore assessed in the 'Entry' and 'Spread' sections.

Q. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals/propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, then comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1–2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and

storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely	**CONFIDENCE**	low

	unlikely moderately likely likely very likely		medium high

Response: N/A

End of pathway assessment, repeat Q. 1.3 to 1.7 as necessary using separate identifier.

Q. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

Q. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30–50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied:

RCP 2.6 (likely range of 0.4–1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

Response: N/A

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of vectors and pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[4] and the provided key to pathways[5].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Q. 2.1. List relevant pathways by which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a vector is the route or mechanism of entry of the species into the environment.

If there are no active vectors or potential future vectors this should be stated explicitly here, and there is no need to answer the questions 2.2–2.8

Pathway name:

a) RELEASE IN NATURE (Fishery in the wild)

b) ESCAPE FROM CONFINEMENT (“Aquaculture” and “Aquarium/garden pond species”)

a) RELEASE IN NATURE (Fishery in the wild)

Q. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional	**CONFIDENCE**	high

Response: There is evidence of both intentional releases to the environment for the purposes of fish stocking and use in extensive (outdoor) aquaculture (Witkowski, 2002; Nowak et al., 2010a, 2010b).

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[4] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[5] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Fish species valued by anglers are often reared in aquaculture facilities and then released into the wild to enhance local fish populations (i.e. stocking).

Q. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this vector from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1–2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: The reputation of *A. nebulosus* as a pest species by anglers makes it less likely to be intentionally released in angling waters, including use in fish stockings, and its use in aquaculture also appears to have reduced dramatically except in certain localised areas. However, the species' natural expansion in Poland has been assisted by intentional introductions carried out by angling associations, fishpond owners, accidental admixture to the stocking material of the other species and using it as live bait (Witkowski, 2002). Similarly, Kapusta et al. (2010) documented a human-assisted release in the Masurian Lake District (Poland) of 40 *A. nebulosus* (probably contaminated with *A. melas*) imported around the year 2000 from the vicinity of Pisz (Masurian Lake District) and deliberately released into Lake Czarne.

Q. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	possible	**CONFIDENCE**	low

Response: Undetected entry into the environment could occur during the stocking of fish from sources where *A. nebulosus* is present if adequate screening of the fish consignment is not implemented.

Q. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	**CONFIDENCE**	medium

Response: Fish stocking is normally undertaken during periods of the year that maximise potential survival, i.e. late winter/early spring, which coincides with the lead into the reproductive period.

Q. 2.6a. How likely is the organism to be able to transfer from the vector to a suitable habitat or host in the environment?

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: Similar to many species, *A. nebulosus* can be spread accidentally or through intentional (but unauthorised) introductions (Nowak et al., 2010a, 2010b). However, *A. nebulosus* is generally regarded as a nuisance species by anglers and is therefore less likely to be intentionally released in angling waters.

Q. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	possible	**CONFIDENCE**	medium

Response: In view of the continued aquaculture use of the species in some parts of the EU, albeit relatively limited, and the propensity of anglers and pet fish owners to release unwanted fish (e.g. Copp et al., 2005a, 2005b), the likelihood of continued releases of this species into locations where it currently does not exist remains moderate.

b) ESCAPE FROM CONFINEMENT (“Aquaculture” and “Aquarium/garden pond species”).

Q. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	Medium

Response: There is evidence of accidental releases of non-native fishes through the stocking of fish (e.g. Copp et al., 2010) and of aquatic plants (e.g. Copp et al., 2017), and the transportation and use of angling gear contaminated by fish eggs (Zięba et al., 2010). Examples of unintentional introduction via inter-basin (trans-watershed) water transfer schemes include the entry of non-native pikeperch *Sander lucioperca* (Wheeler, 1974), and of the native spined loach *Cobitis taenia* from the River Great Ouse basin (East of England) via the Ely Ouse to Essex Transfer Scheme (see Copp & Wade 2006).

Q. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this vector from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1–2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: Similar to many species, *A. nebulosus* could be spread accidentally during fish stocking exercises (Copp et al., 2010; Nowak et al., 2010a, 2010b) or they could escape from aquaculture facilities during extreme hydrological events if the facilities are located on or near rivers (e.g. De Groot, 1985; Walker, 2004). However, the declining interest in the species for both angling and aquaculture suggests that large numbers are unlikely.

Q. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	possible	**CONFIDENCE**	low

Response: Unintentional release during fish stocking is less likely to be detected than accidental escape from aquaculture facilities, given that extreme hydrological events or loss of facility integrity will be noticed and the loss of fish could, theoretically, be quantified. So, overall, the likelihood of detection is moderate.

Q. 2.5b. How likely is the organism to enter into the environment during the months of the

year most appropriate for establishment?

RESPONSE	likely	**CONFIDENCE**	medium

Response: Flooding events in Europe occur normally during late winter/early spring, though in some cases during summer, which coincides with the lead into the reproductive period (spring) or the pre-autumn conditions that allow the fish to escape and adapt to open waters and develop towards reproduction the following spring in shallow waters (Blumer, 1985).

Q. 2.6b. How likely is the organism to be able to transfer from the vector to a suitable habitat or host in the environment?

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: Similar to many species, *A. nebulosus* can be spread accidentally or through intentional (but unauthorised) introductions (Nowak et al., 2010a, 2010b). However, *A. nebulosus* is generally regarded as a nuisance species by anglers and therefore less likely to be intentionally released in angling waters.

Q. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this vector?

RESPONSE	possible	**CONFIDENCE**	medium

Response: In view of the continued aquaculture use of the species in some parts of the EU (e.g. Skolka and Preda, 2010), albeit relatively few and its status questionable (based literature from 1964), the potential for accidental escapes of the fish from aquaculture facilities and for the accidental translocation of this species as a contaminate of authorised fish consignments, the likelihood of continued entry of this species into novel locations remains moderate.

End of pathway assessment, repeat Q. 2.2 to 2.7. as necessary using separate identifier.

Q. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all vectors in current conditions and specify if different in relevant

biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	possible	**CONFIDENCE**	medium

Response: Both intentional and unintentional releases of this species remain possible at this time. However, confidence in this assessment is ‘medium’.

Q. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all vectors in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	possible	**CONFIDENCE**	medium

Response: Near-future climatic conditions are unlikely to modify the intentional use or the accidental release of this species, so scoring is the same as in Q2.8.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Q. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: This species is already established in several member states of the RA area.

Q. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	widespread	**CONFIDENCE**	high

Response: In its native and introduced ranges, *A. nebulosus* inhabits lakes, ponds, reservoirs, rivers and streams as principal habitats, with brackish waters, estuaries and irrigation channels as secondary habitats (Scott and Crossman, 1973; Mandrak, 2009) of which there is a great abundance within the risk assessment area. *Ameiurus nebulosus* can tolerate poor river conditions, and has a wide temperature tolerance. The lack of native competitors and predators could lead to a further range expansion in Europe.

The establishment of *A. nebulosus* following its introduction was likely assisted by its generalist, omnivore diet with feeding aided, even in turbid waters, by its chin barbels (Scott and Crossman, 1973).

This species could invade almost all the inland water surfaces in the EU and especially could become invasive in the southern parts of this region where waters are warmer (Scott and Crossman, 1973).

Q. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	N/A	**CONFIDENCE**	high

Response: There is no evidence available to suggest that the species requires another taxon for any critical stage of its life cycle.

Q. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Summarised in Copp et al. (2016), several authors reported that the decline of *A. nebulosus* in some European countries, e.g. Belgium (Verreycken et al., 2007), Czech Republic (Lusk et al., 2010), Poland (Grabowska et al., 2010) and Hungary (Bódis et al., 2012), has coincided with an increase in the distribution and abundance of *A. melas* in Central and Eastern Europe (Nowak et al., 2010b). These contrasting patterns have led to suggestions that *A. melas* is displacing *A. nebulosus*, but this is not true for Belgium where *A. melas* is not present (Verreycken et al., 2010). This was subsequently reviewed by Béres (2018, p.18): “The research findings confirm the hypotheses that the invasion of *A. melas* started and has not finished yet, and this species invading new habitats gradually replaces *A. nebulosus* not only in the natural waters in Hungary but even all over Europe…”.

These two species have overlapping native distributions in North America (Fuller and Neilson, 2017a, 2017b), so further study is needed in Europe to determine whether or not this pattern of *A. nebulosus* replacement by *A. melas* is simply a coincidental artefact or indicative of *A. melas* displacing *A. nebulosus*.

Q. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	high

Response: There is little information on how predators, parasites or pathogens could affect *A. nebulosus*. Avian predators exist throughout the risk assessment area, but in Iberia the only possible predatory fishes are non-native. By virtue of their strong pectoral and dorsal spines, which can lock into an erect position when threatened, adult *A. nebulosus* are well protected from predation by all but the largest fish predators in their native range in Canada. Although present in juveniles, the spines are less robust making juveniles more susceptible to predation by fishes with a wider range in size. Within its native range, predators include members of the pike family (*Esox* spp.) and pikeperches (*Sander* spp.) (Mandrak, 2009). As such, most piscivorous fishes are unable to predate upon *A. nebulosus* due to the species’ sharp, strong dorsal and pectoral spines (Mandrak, 2009).

Q. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	likely	**CONFIDENCE**	medium

Response: As in many fish species, it is virtually impossible to eradicate *A. nebulosus* once established in a water course or a large water body. However, in small, closed waters (e.g. small lakes or ponds), eradication of fish in general may be possible by chemical means (e.g. rotenone) or by draining down of the water body (Britton et al., 2010b). There is no attempt of eradication of *A. nebulosus* found in the literature but Louette & Declerck (2006) suggest that double fyke nets may potentially be a cost-effective tool for the mass removal of non-indigenous brown bullhead from ponds. The congeneric *A. melas* was eradicated from an isolated pond in Essex, England, using this method (UK Environment Agency, 2014). Other known attempts to eradicate an ictalurid catfish in the risk assessment area include intensive removals of *A. melas* in the Brière Marsh, France, which was only partly successful, probably because of the large area to be fished (Cucherousset et al., 2006a).

Q. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	likely	**CONFIDENCE**	low

Response: No evidence was found to suggest management practices will facilitate the species' establishment, though in some countries inadequate screening of fish consignments (for stocking) could result in the accidental dispersal of non-native fish species including *A. nebulosus* (e.g. Verreycken et al., 2007; Copp et al., 2010).

Q. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	likely	**CONFIDENCE**	medium

Response: Moore et al. (2010) classify *A. nebulosus* as 'high' using the 'hardiness' criterion, which is employed as an indicator of the species' ability to tolerate, survive, or adapt to a wide range of temperatures, pH, salt or freshwater aquatic environments, or the ability to survive out of water for extended periods of time. Indeed, *A. nebulosus* is very tolerant to a range of temperature, oxygen, and pollution conditions that could be limiting for other species. It has been reported that they burrow into the bottom mud to avoid adverse conditions. They seem particularly resistant to domestic and industrial pollution (Scott and Crossman, 1973). They can survive temperatures as high as 36.1 °C. Their upper lethal temperature, under experimental conditions, varied from 28.6–37.5 °C, with acclimation temperatures from 6–36 °C. They survive high carbon dioxide and low oxygen concentrations. In winter, they can live at 0.2 ppm oxygen (Scott and Crossman, 1973). As a result of this tolerance and their bottom habit, *A. nebulosus* is most difficult to eradicate both physically and chemically, the species being less sensitive to the piscicide rotenone than some other species (Ling, 2002).

Q. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very likely	**CONFIDENCE**	high

Response: This species is already established within the RA area. *Ameiurus nebulosus* becomes sexually mature at about 3 years of age (with maximum reported age of 9 years (Kottelat & Freyhof, 2007)), and can lay up to 10,000 eggs although they only breed once a year. Survival is increased throughout the egg/juvenile stage due to maternal protection, which usually lasts for the first 29 days after hatching (Guth, 2011). Additionally, Moore et al. (2010) have stated that all *Ameiurus* species, except *A. serracanthus*, present a moderate population growth, according to the criterion ‘resilience’, which indicates the rate of population doubling as an indicator of the rate of potential population growth.

Q. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	very likely	**CONFIDENCE**	high

Response: The species’ tolerance of a vast array of water quality variables enhances its ability to adapt to and live in a range of freshwater habitats. This is apparent in the species’ establishment in various global locations outside its native range, including Europe, Caribbean islands (i.e. Puerto Rico), and New Zealand (e.g. Barnes and Hicks, 2003; Neal et al., 2009).

Q. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	possible	**CONFIDENCE**	low

Response: No evidence was found to suggest that low genetic diversity would reduce this species' chances of establishment.

Q. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species that cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	possible	**CONFIDENCE**	Medium

Response: As mentioned here above, *A. nebulosus* inhabits lakes, ponds, reservoirs, rivers and streams, brackish waters, estuaries and irrigation channels, and it is able to tolerate, survive or adapt to a wide range of temperatures, pH, salt or freshwater aquatic environments (Scott and Crossman, 1973; Moore et al., 2010). As such, failure to establish is unlikely, but if establishment is not achieved, then persistent as a casual is very likely, though a casual fish is not likely to persist beyond eight or nine years (max. lifespan is about 9 years (Kottelat & Freyhof, 2007)).

Q. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very likely	**CONFIDENCE**	medium

Response: Both the native and EU ranges of this species encompass five climate type zones (Peel et al., 2007), with four of these shared by the native and EU ranges (Cfa, Dfa, Dfb, Dfc), as such establishment in the risk assessment area, even in other parts where it is not yet established (see QA9a), is very likely under current climatic conditions.

Q. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be

provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.
With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very likely	**CONFIDENCE**	medium

Response: With climate change affecting water temperatures, an increase in the latter is likely to facilitate the establishment of this species, assuming a similar response to warmer temperatures as observed in its close congener *A. melas* (Britton et al., 2010a). Britton et al. (2010a) ran a comparison of mean Climatch scores between 2009 and 2050 for *A. melas* in the UK. *Ameiurus melas* has an increased climate match with the source region in 2050 when compared with 2009. This species is likely to benefit from climate warming in England and Wales, this prediction was then tested using water temperature modelling. One can expect that similar benefit is true for regions between 50° and 55° N as modeled by Britton et al. (2010a). As such, it is likely to establish in more areas where previously the water temperature would be too low to reproduce. This would facilitate establishment in regions currently with a colder climate, such as the U.K. (Britton et al., 2010a), Poland and Germany, where the species is already established (Grabowska et al., 2010; Wolter et al., 2000). The increase in temperature would allow *A. nebulosus* to spread and establish more widely into all biogeographic regions except probably the Alpine.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic "spread" via release or escape ("jump-dispersal"), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Q. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	moderate	**CONFIDENCE**	medium

Response: In European waters, natural spread of *A. nebulosus* could occur within and between countries via water courses (Panov et al., 2009; Nowak et al., 2010b). However, *A. nebulosus* has been established in several European countries for over a century, and natural dispersal appears to be slow. For example, despite its presence in Flanders (Belgium) since 1871, the distribution of *A. nebulosus* is still restricted to the northeastern part of this region only (Verreycken et al., 2007). Also, in the Czech Republic, *A. nebulosus* occurs only locally, without showing any tendency towards spreading (Lusk et al., 2010). Recently, in Greece, a self-sustained population of *A. nebulosus* was discovered and it is supposed that the fish was introduced from Bulgaria through the transboundary waters of the Strymon River (Barbieri et al., 2015). Dense populations have formed in standing waters only, with movements of adult *Ameiurus* species tending to be localised, which has also been observed in its close congener, *A. melas* (Bouvet et al., 1982, 1985). After hatching, the young of both species form dense ball-shaped shoals that follows the female around for approximately a month prior to local dispersal. Therefore, this species is less likely to spread rapidly than some other fish species from other genera. Overall, there is relatively limited information about the distribution of *A. nebulosus*, in particular the status of populations in Central and Eastern Europe (Rechulicz and Płaska, 2018).

Q. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread

and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	moderate	**CONFIDENCE**	medium

Response: As reported here above, *A. nebulosus* can be spread accidentally or through intentional (but unauthorised) introductions (Nowak et al., 2010a, 2010b), but because it is generally regarded as a nuisance species by anglers, it is less likely to be released intentionally by anglers. This would be a reversal of past practices in Poland, where intentional introductions continued up to about the year 2000 through the angling vector (Witkowski, 2002; Kapusta et al., 2010).

Q. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway names: a) CORRIDOR - Interconnected waterways/basins; and b) RELEASE IN NATURE - Other intentional release.

a) CORRIDOR - Interconnected waterways/basins

Q. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: Secondary, natural dispersal of an organism following its release is most likely to be an unintentional consequence of the entry, both the intentional release and the unintentional escape, of organisms into a new drainage basin (assessed in the ‘Probability of Entry’ section here above).

Q. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	possible	**CONFIDENCE**	medium

Response: Following the intentional release or the unintentional escape of organisms into a new drainage basin (assessed in the ‘Probability of Entry’ section here above), the number of individuals involved in secondary dispersal within the new drainage basin would depend on the numerical size of that basin’s source population and on the connectivity between the point source and the remainder of the drainage basin. However, it is moderately likely that there would be sufficient numbers dispersing over the course of the year, given the likelihood of floods/spates during certain seasons, which increase connectivity (e.g. Copp, 1989; Amoros and Bornette, 2002).

Q. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Natural dispersal does not involve storage, but survival during natural spread (i.e. ‘transport’) along water courses and canals is very likely, with subsequent reproduction possible. Indeed, the reproduction of some fish species is triggered and facilitated by inundation of the flood plain (e.g. northern pike *Esox lucius*). In *A. nebulosus*, spring-time migrations upstream are known to occur in the native range (Sakaris et al., 2005) and also in its introduced New Zealand range (Dedual, 2002).

Q. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Unless there is a specific monitoring programme and rapid-response protocol that targets pest fish species, it is very likely the organism would survive existing management practices because their dispersal along water ways will not be detected. There is a multitude of bibliographic sources that demonstrate the difficulty of detecting rare fish species in running waters.

Q. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: Unless there is a species-specific sampling programme that involves conventional and environmental DNA detection methods, the species' spread along water ways will be detected only by anglers perhaps and/or incidental encounters during routine monitoring.

Q. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	likely	**CONFIDENCE**	medium

Response: Most water ways involve proximity to some form of flood plain that contains still waters, which are the preferred habitat of *A. nebulosus* (Scott and Crossman, 1973), and most EU water courses are subjected to floods and spates that result, even in regulated systems, in the overflow of the water course into the adjacent flood plain.

Q. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	slowly	**CONFIDENCE**	medium

Response: In European waters, natural spread of *A. nebulosus* could be within and between countries via water courses (Panov et al., 2009; Nowak et al., 2010b). However, as described here above, *A.*

nebulosus is a relatively sedentary species (Millard et al., 2009), with evidence for natural dispersal suggesting slow spread (e.g. Verreycken et al., 2007; Lusk et al., 2010).

b) RELEASE IN NATURE - Other intentional release.

Q. 4.3b. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	**CONFIDENCE**	high

Response: Secondary, human-assisted dispersal of an organism, following its entry into a previously-unoccupied drainage basin, whether by intentional release and via unintentional escape (assessed in the ‘Probability of Entry’ section here above), can result in intentional human translocation of that organism within the new drainage basin (e.g. Copp et al., 2005b).

Q. 4.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	moderately likely	**CONFIDENCE**	low

Response: This will depend upon how many fish were released (or escaped) into the ‘point of origin’, so confidence is low.

Q. 4.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Human-assisted storage and transport is normally undertaken with the intention of maximum survival of the organism, so as to achieve the intended purpose at the point of new release. So, it is very likely that the organism will survive the relatively short translocation within the same drainage basin for release to a previously-uninhabited part of that drainage basin. Reproduction is highly unlikely during such short transport and/or storage.

Q. 4.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very likely	**CONFIDENCE**	medium

Response: With regard to releases for the purpose of stocking the fish into a new location within the same drainage basin, whether authorised or not, management of the fish stocks by the person(s) undertaking the release of fish can be assumed to be with the intent of the species' survival. In the case of an unauthorised release, existing management practices of the government authorities are unlikely to affect the survival of the translocated fish except if they disperse out of the stocked (intended) location into adjacent waters that are subject to control of government agencies. As such, survival of existing management practices is highly likely.

Q. 4.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	unlikely	**CONFIDENCE**	high

Response: Unless there is a species-specific sampling programme that involves conventional and environmental DNA detection methods, the species' spread along water ways will be detected only by anglers perhaps and/or incidental encounters during routine monitoring. In the case of unauthorised releases within the same drainage basin, these are likely to be clandestine and therefore unlikely to be detected.

Q. 4.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very likely	**CONFIDENCE**	high

Response: In the case of intended release to a new part of the same drainage basin, this is assumed to be into suitable habitat, so as to achieve the purpose of the stocking, whether authorised or not.

Q. 4.9b. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	slow	**CONFIDENCE**	medium

Response: Unlike natural dispersal of *A. nebulosus*, which is considered to be slow (e.g. Verreycken et al., 2007; Lusk et al., 2010), translocations by humans would normally be at least moderate if the species is of interest. However, this species is generally considered to be a nuisance/pest (Nowak et al., 2010b, Grabowska et al., 2010), so translocation of this species, whether authorised or not, is likely to be slow.

End of pathway assessment, repeat Q. 4.3 to 4.9. as necessary using separate identifiers.

Q. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	difficult	**CONFIDENCE**	high

Response: It is well known that containment and eradication of fish, once in a water course, is difficult, if not impossible (e.g. Tyus and Saunders, 2000). Basically, the likelihood of containing and extirpating a fish species from a water course is inversely related to the size (width, depth, water discharge) of the water course. Whereas, containment and potential eradication is possible in smaller, enclosed waters (Britton et al., 2010b).

Q. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	slow	**CONFIDENCE**	medium

Response: As described here above, *A. nebulosus* is a relatively sedentary species, with evidence for natural dispersal suggesting slow spread (e.g. Verreycken et al., 2007; Lusk et al., 2010).

Q. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	slow	**CONFIDENCE**	medium

Response: In their native range, telemetry studies have demonstrated a preference in *A. nebulosus* for warmer waters (Kelso, 1974; Richards and Ibara, 1978; Crawshaw et al., 1982; Sakaris et al., 2005), which could suggest that an increase in mobility may be expected under warmer climate conditions. However, the evidence for a sedentary existence (e.g. Sakaris et al., 2005; Millard et al., 2009), which appears to be shared by its close congener, *A. melas* (Bouvet et al., 1985), suggests that any such increased mobility is likely to be modest. Most water ways involve proximity to some form of flood plain that contains still waters, the preferred habitat of *A. nebulosus* (Scott and Crossman, 1973), and the incidence (frequency and intensity) of extreme hydrological variations is projected to increase in many EU water courses under future climate conditions. This would result, even in regulated systems, in the overflow of the water course into the adjacent flood plain, thus enhancing the dispersal of *A. nebulosus*, though not as rapidly as species of greater, natural migratory inclination.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1–5.5 relate to biodiversity and ecosystem impacts, 5.6–5.8 to impacts on ecosystem services, 5.9–5.13 to economic impact, 5.14–5.15 to social and human health impact, and 5.16–5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Q. A.7)

Biodiversity and ecosystem impacts

Q. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	major	**CONFIDENCE**	medium

Comment: *A. nebulosus* is known to have extirpated the *Gasterosteus* species pair from a lake in British Columbia, Canada (Hatfield, 2001), the Pacific Northwest being an area where there are several lakes where the only native fish species is *Gasterosteus aculeatus*, which is present in distinct limnetic and littoral forms. See also Voller and McNay (2007).

Ameiurus nebulosus introductions may also lead to changes in aquatic communities through their use of resources (food or space), with competition possible, though dietary overlap is more easily demonstrated as Collier et al. (2018) pointed out for New Zeeland. *Ameiurus nebulosus* is a scavenger species as well as predacious, locating their prey in the substratum through the use of their sensory barbels (Scott and Crossman, 1973), with predation on small fishes, invertebrates or other small food items known in populations of both the native (e.g. Keast, 1985) and introduced (e.g. Guti et al., 1991; Declerck et al., 2002; Barnes and Hicks, 2003) ranges. Of particular concern is the potential for altering ecosystems in New Zeeland from macrophyte-dominated clear water states and devegetated, turbid states, given their benthic behaviour (Schallenberg and Sorrell, 2009).

An omnivore, *A. nebulosus* feed mostly at night and eating benthic organisms that occur within freshwaters: waste, molluscs, immature insects, terrestrial insects, leeches, crustaceans, worms, algae, plant material, fishes and fish eggs. Juvenile *A. nebulosus* (30–60 mm total length) prefer chironomid larvae, ostracods, amphipods, mayflies and other small aquatic invertebrates (Scott and Crossman, 1973)

Moore et al. (2010), in their review of the species for Australia's management of ornamental fishes, included all 7 *Ameiurus* species in the 'high' risk category. Mandrak (2009), in the CABI datasheet, reported that the main mechanism for *A. nebulosus* is competition: monopoly of resources, interaction with other invasive species and predation. The main outcomes are altered trophic level, damaged ecosystem services, ecosystem change/habitat alteration, and modification of natural benthic communities, negative impacts on aquaculture/fisheries, reduced native, biodiversity, threat to/loss of endangered native species. Among the affected species by predation indicated by Mandrak (2009) are Oregon spotted frog *Rana pretiosa* in California, Oregon and Washington and Shasta crayfish *Pacifastacus fortis* in California. The genus *Gasterosteus* in British Columbia is affected by competition with and predation by *A. nebulosus*, which extirpated the *Gasterosteus* species pair, the only native fish in the lake (Hatfield, 2001).

Chile reported adverse effects on native fish communities following the establishment of *A. nebulosus,* however the species current status and locations in that country are listed as 'somewhere in Chile' (Iriarte et al., 2005).

In New Zealand, where the species is non-native (Schallenberg and Sorrell, 2009), the main prey in Lake Taupo of adult *A. nebulosus* was reported to be freshwater crayfishes (Global Invasive Species Database, 2019). In shallow New Zealand lakes, a study of trophic overlap between *A. nebulosus* and native shortfin eel *Anguilla australis* found that *A. nebulosus* has higher potential to influence the *Anguilla australis* nutrition than vice versa, or that a broad trophic niche occupied by *A. australis* provides resilience to the effects of overlapping consumption patterns with invasive omnivores (Collier et al., 2018).

Q. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	major	**CONFIDENCE**	low

Comment: Savini et al. (2010), in the review of the impacts caused by the most important 25 aquatic alien species intentionally introduced in European waters, recorded ten references of potential impacts by *A. nebulosus*: bioaccumulation (storage and magnification toxic substances in tissues), community dominance (species causing quantitative changes in community structure in becoming the dominant species), competition (for food or for space with native species) and predation (predatory activity on native species). However, the references for these citations are not provided either in Savini et al. (2010) nor in the contract report (Occhipinti Ambrogi et al., 2008) from which that article was derived.

In the species' introduced European range, there is evidence of potentially negative impacts by *A. nebulosus* such as predation on native species (e.g. Guti et al., 1991; Declerck et al., 2002), but there has in fact been few studies of the species' impacts (Copp et al., 2016). Therefore, the confidence for this question in Low. Most studies have simply examined the species' diet upon which inferences of threats to native species have been made. However, predation on young-of-the-year fish by *A. nebulosus* can impact on threatened native fishes, such as the species' predation of young-of-the-year crucian carp *Carassius carassius* (Guti et al., 1991), a species threatened in many parts of its native Continental European range (Tarkan et al., 2016). Whether or not *A. nebulosus* predation on *C. carassius* is a factor in its decline remains unknown, and in the case of Hungary, *A. nebulosus* has been in decline since the 1950s, so its impact there would be very localised (Bódis et al., 2012).

This omnivorous fish species can form very dense populations and is able to dominate freshwater fish communities. Diet of large-sized *A. nebulosus* has been found to consist almost exclusively of juvenile fishes. Although there is some concern about its strong competitive and predation ability, the lack of scientific study (Rechulicz and Płaska, 2018) makes it difficult to find hard evidence of species displacement or ecosystem disruption (Anseeuw et al., 2007; Skolka & Preda., 2010).

In order to assess environmental and economic impact of alien and invasive fish species in Europe using the generic impact scoring system, Van der Veer and Nentwig (2015) calculated the impact points obtained by the generic impact scoring system in six environmental impact categories for *A. nebulosus* (herbivory, predation, competition, transmission of diseases, hybridisation and ecosystem alteration). Comparing the mean score for 40 established alien fish species, five of the scores for environmental impact (except hybridisation) were greater in the case of *A. nebulosus*.

In Czech Republic, the negative impact of *A. nebulosus* is considerably limited due to its local occurrence and heavily decreased numbers (Luks et al., 2010). On the other hand, there exist some local (non-scientific) reports in the Czech Republic that during early spring, specimens of the genus *Ameiurus* can eliminate native salamanders through predation. Moreover, Šukalo et al. (2012) warns of the damage inflicted in specimens of the genus Natrix in Bosnia and Herzegovina by the stiff spiny rays of the dorsal and pectoral fins of *A. nebulosus* and other non-native species that grass snakes feed on.

Q. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	major	**CONFIDENCE**	medium

Comment: This species is able to establish across a wide range of climatic zones, so the predicted warmer conditions for virtually all of the EU is unlikely to modify the likely magnitude of impacts by *A. nebulosus* in the future.

Q. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	moderate	**CONFIDENCE**	medium

Comment: According to the EU's Water Framework Directive (WFD), the status of all European rivers and streams must be assessed and classified into five predefined levels of ecological status (bad, poor, moderate, good and high) based on four biotic elements (diatoms, macroinvertebrates, macrophytes and freshwater fish) (Hermoso et al., 2010). Any decline in native species, and/or an increase in non-native species, is related with a poor ecological status, so it has influence in this classification. However, the manner with which non-native species presence affects WFD classification status is not consistent across the EU. In view of the species' potential to generate turbidity, this could affect the ecological status of smaller water bodies, but these are not covered by the WFD, so the magnitude of impact on status will depend upon the size of the water body, its conservation status, and national legislation regarding non-native species.

Two examples of the presence of *A. nebulosus* in sites of nature conservation value include: Poland, where *A. nebulosus* is present in the Landscape Park "Dolina Baryczy" (the Barycz Valley), which is listed amongst the "Living Lakes" for protection under the Ramsar Convention as well as under the European nature protection network Natura 2000. One protective action in the active protection area is the careful elimination of all *A. nebulosus* specimen obtained during yield (Tokarczyk-Dorociak et al., 2016). In Romania, the species occurs in Iron Gates Natural Park but no information on impacts is provided (Ciocănea et al., 2016).

Q. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats

- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	moderate	**CONFIDENCE**	low

Comment: If the current climate carries on getting warmer, this suggests that this species could spread more rapidly than the current ‘slow’ spread, and this species could have a greater adverse impact on native species and aquatic ecosystems that are the subject of conservation interest and legislative protection.

Ecosystem Services impacts

Q. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal	**CONFIDENCE**	low

Comment: Although *A. nebulosus* is mentioned in papers that discuss non-native species impacts on ecosystem services (e.g. Chadderton, 2001; Gozlan, 2010), no evidence of ecosystem services impact is presented. However, in water bodies used by anglers, their perception of the angling value may be reduced by the species’ presence (unpublished statements from discussions with anglers), but scientific studies of the impacts on ecosystem services (e.g. decline in use of water bodies due to invasive fish presence) are lacking.

Q. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Q. 5.6.

RESPONSE	N/A	**CONFIDENCE**	-

Comment: No published evidence has been found that would allow to answer this question.

Q. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Q. 5.6.

RESPONSE	N/A	**CONFIDENCE**	-

Comment: No published evidence has been found that would allow to answer this question.

Economic impacts

Q. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minor	**CONFIDENCE**	low

Comment: The economic impact assessment by Van der Veer G. and Nentwig (2015) indicated ‘0’ impacts for *A. nebulosus*, which is likely to have been based on the absence of information rather than hard evidence, given that no studies are known to have been undertaken on the economic losses associated with *A. nebulosus* (Mandrak, 2009) – and indeed such studies on any non-native freshwater fish species are rare, and these have dealt entirely with management costs (e.g. Britton et al. 2010). In certain cases of wild establishment, A. *nebulosus* introductions have the potential to hinder local commercial and sport fisheries through competition with target species (Allen and Godard, 2015; Mandrak, 2009).

Q. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minor	**CONFIDENCE**	low

Comments: A lack of published evidence on this (Mandrak, 2009) suggests that impacts are sufficiently minimal as not to warrant study. The only potentially relevant information is that for the species close congener, *A. melas*, which Gallardo and Aldridge (2013) included in the list of 12 aquatic species potentially causing greatest ecological and economic harm to Great Britain and Ireland. However, there was only one confirmed population of *A. melas* in Great Britain and Ireland – it was located in an isolated, private land, location a long distance from any connecting water course, and that population was eradicated in 2014 (UK Environment Agency, 2014).

Q. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Q. 5.10.

RESPONSE	N/A	**CONFIDENCE**	-

Comments: No published evidence has been found that would allow to answer this question.

Q. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minor	**CONFIDENCE**	medium

Comments:

There is some mention in the literature of *A. nebulosus* eradications (e.g. Chadderton, 2001), or attempted eradications of its close congener, *A. melas* (Cucherousset et al., 2006a), the costs of these initiatives are not given.

It can be assumed that, although widely spread in the risk assessment area, overall no systematic effort has been undertaken to manage the species across the RA area.

The economic costs of eradication of *A. nebulosus* could be relatively modest or very high, depending upon the extent of the species' spread, the size of the water bodies it invades, etc. For example, the cost of the operation to remove its close congener, *A. melas*, from the small pond in Essex (UK Environment Agency, 2014) was ≈ £5000–£10000 (≈ €5400–€10900), including personnel costs (UK Animal and Plant Health Agency, personal comm.), however all of the angling club's fish were lost due to the rotenone treatment.

Similar range of costs are reported by other invasive fish eradications in the U.K., e.g. for topmouth gudgeon *Pseudorasbora parva*, it was demonstrated that the costs of eradication increase with increasing larger waterbody size (e.g. Britton et al., 2010b, 2011), but on average £20K GBP per hectare (≈ €22k/ha) (Britton et al., 2008).

Another example is the cost of eradicating northern snakehead *Channa argus* from a small pond in Crofton, Maryland (U.S.A.), which was estimated to be $110k USD (≈ €100k). This included personnel time for planning meetings, field application of the piscicide, and disposal of the dead fish (Courtenay and Williams, 2004). In 2010 alone, the US federal government committed $78.5 million in investments to prevent the introduction of Asian carp to the Great Lakes, where they would threaten Great Lakes fisheries and could negatively impact remaining populations of endangered or threatened aquatic species (U.S. Fish and Wildlife Service, 2012).

Q. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Q. 5.12.

RESPONSE	minor	**CONFIDENCE**	low

Comments: The costs identified in the comments to Q. 5.12 would be expected to increase should the species spread more widely, as is suggested in the 'Spread'.

Social and human health impacts

Q. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal	**CONFIDENCE**	medium

Comments: A painful wound can be inflicted by the sharp spines in the fins of *A. nebulosus* if they are not handled carefully. Toxins released by the fish contribute to the pain of the wound (Verreycken, pers. experience, Global Invasive Species Database, 2019). In some waters, *A. nebulosus* have been found to contain elevated levels of contaminants (Arcand-Hoy and Metcalfe, 1999; Pinkney et al., 2001; Savini et al., 2010), which could pose a problem in cases where this species is taken from contaminated waters and used as a food stuff.

Q. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	Minimal	**CONFIDENCE**	low

Comments: As mentioned above, painful wounds can be inflicted by the sharp spines in the fins of *A. nebulosus* if they are not handled carefully, and *A. nebulosus* have been found to contain elevated levels of contaminants, which poses a risk in cases where this species is taken from contaminated waters and eaten.

Other impacts

Q. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	moderate	**CONFIDENCE**	Medium

Comments: *Ameiurus nebulosus* is a susceptible species to host *Aphanomyces invadans* related to the Epizootic ulcerative syndrome. Scott and Crossman (1973) describe the following parasites known from within the species, which have the potential to infect recipient fish communities following *A. nebulosus* introductions: Protozoa, Trematoda, Cestoda, Nematoda, Acanthocephala, leeches, Mollusca and Crustacea.

Moldowan et al. (2015) noted that common snapping turtle *Chelydra serpentina* in Ontario (Canada) do not necessarily discriminate against *A. nebulosus* as prey item despite the risk posed by their defensive pectoral spines. As noted by Sismour et al. (2013), the effectiveness of catfish pectoral spines as an anti-predator defense depends on the relative predator–prey size and on predator aggressiveness.

Ameiurus nebulosus hosts *Carnobacterium* (*piscicola*) *maltaromaticum* in the kidney, and may be a carrier (Buller, 2014). *Ameiurus nebulosus* hosts *Edwardsiella ictaluri*, which is related to an infection in the brain, with systemic dissemination and localisation in the visceral organs and musculature and cutaneous ulcers. This parasite is related to enteric septicaemia of catfish (ESC) and edwardsiellosis (Buller, 2014). It is possible for rainbow trout *Oncorhynchus mykiss* to be a host for this pathogen. With this finding it could be possible for this pathogen to be more widespread if *A. nebulosus* brings the pathogen in to a water body with *O. mykiss* present (Keskin et al., 2004). Moreover, it hosts *Edwardsiella tarda* related to septicaemia, focal suppurative or granulomatous lesions and cutaneous ulcerations, and to edwardsiellosis. It is an opportunistic infection (Buller, 2014). This pathogen can be fatal for humans although it is relatively rare at <5% (Janada & Sharon, 1993).

Q. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A	**CONFIDENCE**	-

Comments: None have been encountered in the literature search.

Q. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	major	**CONFIDENCE**	low

Comments: It is not well known how predators, parasites or pathogens could affect *A. nebulosus* and any information available indicates there are no other organisms that would control it naturally. At least in Spain, the only possible fish predators will also be exotic. Within the species native and introduced European ranges, predators include members of the pike family (*Esox* spp.) and pike perches (*Sander* spp.) (Mandrak, 2009). However, some piscivorous fishes are unable to predate *A. nebulosus* due to their sharp, strong dorsal and pectoral spines that may lock into an erect position when predated upon (Mandrak, 2009). Although present in juveniles, the spines are less robust making juveniles more susceptible to predation by fishes with a wider range in size. These spines, combined with the species' nocturnal feeding regime, make *A. melas* an uncommon prey item for most fish species. However, some piscivorous birdes, such as cormorants and herons, as well as some turtle species, will occasionally consume the young and small adults of ictalurid catfishes (Allen and Godard, 2015).

Q. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	moderate	**CONFIDENCE**	low

Comments: The scarcity of evidence of impacts in the risk assessment area, mainly due to a lack of such studies, makes it difficult to assess the species current impacts. In view of the species' relatively limited current, localised distribution, with some evidence of declines in European range, the overall impacts in the RA area are likely to be moderate, being minimal-to-minor in some areas and perhaps moderate-to-major in specific areas.

Q. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	moderate	**CONFIDENCE**	low

Comments: The scarcity of evidence of impacts in the risk assessment area, mainly due to a lack of such studies, makes it difficult to assess the species future impacts. However, the evidence of declines in the species' European range, which contrasts the potential enhancement of establishment potential under conditions of climate warming, suggests that the overall impacts in the RA area could be moderate, being minimal-to-minor in some areas and moderate-to-major in areas where the species is particularly invasive.

RISK SUMMARIES

	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction*	very unlikely	medium	*Ameiurus nebulosus* is already present in several EU countries, but there are no active introduction vectors. Previous vectors include introductions via fish stocking (for angling/sport purposes) and for aquaculture.
Summarise Entry*	possible	medium	Unauthorised introductions of *A. nebulosus* by anglers have occurred within and between EU countries in the past and are likely to continue, though perhaps less frequently due to the declining interest for aquaculture and the increasing anglers' view of the species as a pest.
Summarise Establishment*	very likely	medium	*Ameiurus nebulosus* is established in several EU countries, but there is some evidence of populations declining and/or disappearing. Circumstantial evidence has suggested this decline has been related to the spread of its close congener, *A. melas*, but hard evidence for this is lacking and this latter species has declined in at least one country. The species can inhabit a wide range of freshwater ecosystem, as well as mildly brackish waters, and therefore could potentially adapt easily to the climatic conditions in some countries where it currently does not exist, if allowed to be translocated. The species is tolerant of poor water quality, including contaminants, and a wide range of water temperatures. The lack of native competitors and predators in some locations could lead to a further range expansion in Europe, though in other locations native predators (pikes, pikeperches) are present.
Summarise Spread*	slow	medium	Dispersal of *A. nebulosus* has been relatively slow, which is due

			to the species mainly sedentary character, though seasonal migrations of short distances up and downstream have been demonstrated in telemetry studies, both in the species native and non-native (New Zealand) ranges. Translocations of *A. nebulosus*, following its release into a new drainage basin, are possible (e.g. by anglers) but this is expected to decline in importance due to the species perception by anglers as a nuisance species (Hubble, 2011).
Summarise Impact*	moderate	medium	There is some information on adverse ecological impacts by *A. nebulosus* outside its native range, including modification of water quality and ecosystem character, but there are no known studies of the species' socio-economic or ecosystem services impacts. Areas of high nature conservation value, including National Parks, are at particular risk of economic, environmental and social negative impacts. The species is known to be associated with some diseases, which could affect native species, but mass fish kills due to *A. nebulosus*-borne diseases were not found. Published studies that report on the economic costs associated with managing this species were not found, but information is available from the U.K., where its close congener, *A. melas*, has been eradicated from a single location (UK Environment Agency, 2014), and the mean cost per unit area be estimated (Britton et al., 2008) Although there is evidence of hybridisation between species of the same family (Ictaluridae), there are no closely-related species native to the EU so hybridisation is extremely unlikely if not impossible.

			A painful wound can be inflicted by the sharp spines in the fins of *A. nebulosus* if they are not handled carefully. Toxins released by the fish contribute to the pain of the wound. These spines reduce the species' risk of predation but there are at least two taxon groups (pikes and pikeperches) that can prey on *A. nebulosus*.
Conclusion of the risk assessment (overall risk)	moderate	medium	The species' distribution includes several member states of the risk assessment area but populations are localised and there is some evidence of decline of the species across its EU range. Introduction is unlikely due to vectors and pathways having generally ceased to operate, but intentional and accidental releases of fish into open waters and translocations from existing populations continue to pose a moderate level of risk. Entry into open waters is probably the main means of dispersal of this otherwise sedentary species, which means that natural spread is slow and more rapid dispersal is primarily human assisted. The fact that the species has established in various member states of the risk assessment area evinces its relatively high risk of establishment. Potential impacts include increased turbidity, especially in smaller water bodies and potential decreases in the ecosystem services (mainly angling), though no studies of economic loss produced by *A. nebulosus* were found. Other potential impacts include the transmission of fish diseases to some native fish species (e.g. European catfish *Silurus glanis*).

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	Yes	Yes	Yes	Yes	-
Belgium	Yes	Yes	Yes	Yes	-
Bulgaria	Yes	Yes	Yes	Yes	-
Croatia	Yes	Yes	Yes	Yes	-
Cyprus	-	-	Yes	Yes	-
Czech Republic	Yes	Yes	Yes	Yes	-
Denmark	Yes	Yes	Yes	Yes	-
Estonia	-	-	Yes	Yes	Yes
Finland	Yes	Yes	Yes	Yes	-
France	-	-	Yes	Yes	-
Germany	Yes	Yes	Yes	Yes	Yes
Greece	Yes	Yes	Yes	Yes	-
Hungary	Yes	Yes	Yes	Yes	-
Ireland	-	-	Yes	Yes	-
Italy	Yes	Yes	Yes	Yes	-
Latvia	-	-	Yes	Yes	-
Lithuania	-	-	Yes	Yes	-
Luxembourg	-	-	Yes	Yes	-
Malta	-	-	Yes	Yes	-
Netherlands	Yes	Yes	Yes	Yes	-
Poland	Yes	Yes	Yes	Yes	Yes
Portugal	-	-	Yes	Yes	-
Romania	Yes	Yes	Yes	Yes	Yes
Slovakia	Yes	Yes	Yes	Yes	-
Slovenia	Yes	Yes	Yes	Yes	-
Spain	-	-	Yes	Yes	-
Sweden	-	-	Yes	Yes	-
United Kingdom	Yes	-	Yes	Yes	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	-	Yes	-
Atlantic	Yes	Yes	Yes	Yes	-
Black Sea	-	-	Yes	Yes	-
Boreal	Yes	-	Yes	Yes	-
Continental	Yes	Yes	Yes	Yes	-
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian	Yes	Yes	Yes	Yes	-
Steppic	-	-	Yes	Yes	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea	-	-	-	-	-
Black Sea	-	-	-	-	-
North-east Atlantic Ocean	-	-	-	-	-
Bay of Biscay and the Iberian Coast	-	-	-	-	-
Celtic Sea	-	-	-	-	-
Greater North Sea	-	-	-	-	-
Mediterranean Sea	-	-	-	-	-
Adriatic Sea	-	-	-	-	-
Aegean-Levantine Sea	-	-	-	-	-
Ionian Sea and the Central Mediterranean Sea	-	-	-	-	-
Western Mediterranean Sea	-	-	-	-	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[6]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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[6] Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al., 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition,*

			predations, spread of disease etc.)
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[7]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*

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[7] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*

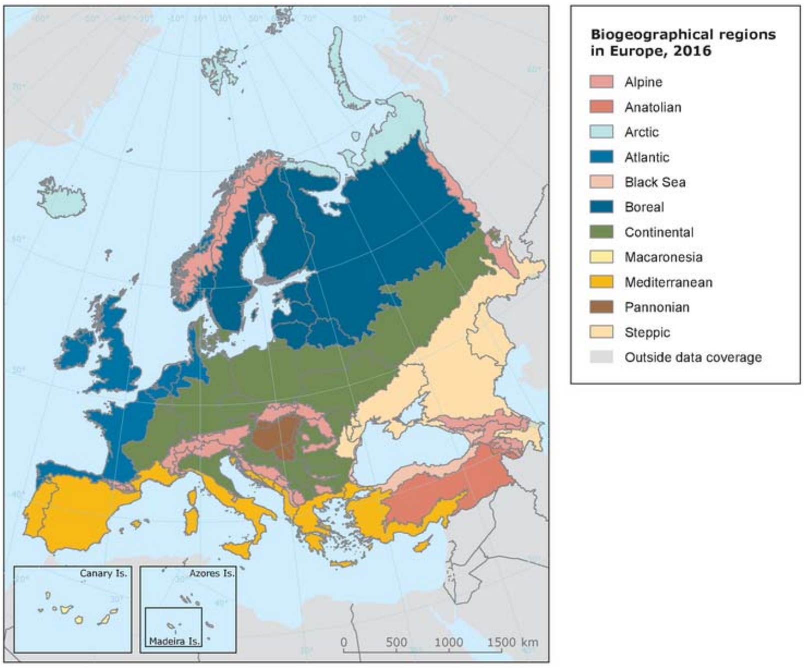
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

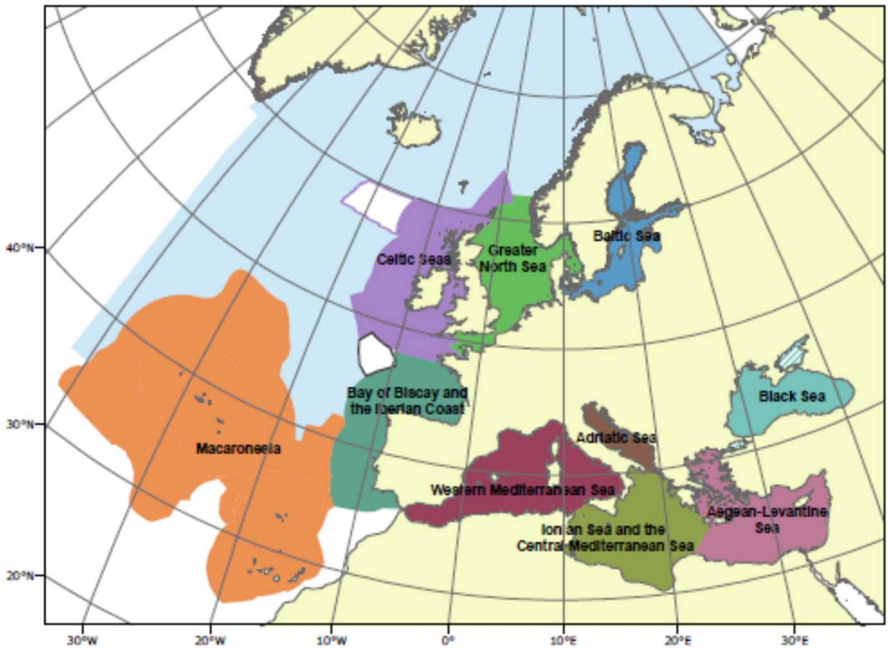
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	*Ameiurus nebulosus* (Lesueur, 1819)
Species (common name)	**Brown bullhead**
Author(s)	**H. Verreycken, L. Aislabie, G.H. Copp**
Date Completed	**23 October 2019**
Reviewer	**Sebastian Kozic, Philip Haubrock**

Summary [1] Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.
Ameiurus nebulosus is already widespread in the EU and there are no relevant pathways of introduction and entry in the RA area. Previous pathways for introduction into Europe are not considered relevant anymore. Unauthorized introduction by anglers is estimated to be the main origin for new within- and between-member states introductions and spread. The adoption and enforcement of appropriate legislation and codes of best practice could reduce the likelihood of introduction. The most cost-effective way of preventing intentional introductions is to raise public awareness of the problems associated with the establishment of *A. nebulosus.* It will educate people on the impacts of invasive alien species (IAS) and, if people become more educated, they are less likely to release them in the environment and more likely to report IAS when they see them. Early detection of the species in new infested sites is very hard due to the areas this species inhabits and their benthic, hidden life. The use of eDNA is a new promising tool here. Additional reporting from recreational fishermen of new findings of this fish would facilitate the targeted monitoring for *A. nebulosus*. Although fish surveys (e.g. for the WFD) would be a continuous way of early detection, it is a very expensive measure and it is not guaranteed that the fish will be detected. Rapid eradication of the species is dependent on where and at what stage it is found. The potential to eradicate or control *A. nebulosus* populations depends on dispersal location and the level of establishment. In small enclosed water bodies, piscicides (rotenone) could be effective in eradicating populations, but legal constraints on the use of piscicides may hamper their success in the EU. Mechanical removal using fyke nets, electrofishing and other fishing gear may be successful in managing ictalurid catfish populations in

small confined areas. Targeted angling can also be a part of the removal exercise of this species.

There is very limited literature in relation to costs associated with prevention, eradication and management programmes for *A. nebulosus*.

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)**[3]	**Level of confidence**[4]
Methods to achieve prevention [5]	**Managing the pathways:** *Ameiurus nebulosus* was introduced in Europe in the late 1800s and is already widespread here. At present, there are no active vectors of introduction but entry to the environment may continue to occur (unauthorised) via historical vectors, i.e. angling/sport purposes (within and between members states), aquarium release, and aquaculture – though all are in decline. The adoption and enforcement of appropriate legislation and codes of best practice could reduce the likelihood of introduction to the EU and entry to the environment.	Enforcement of appropriate legislation and codes of best practice would be an effective means of limiting the risk of intentional spread of *A. nebulosus* by anglers between and within member states. There is no information available about the costs and the equipment or infrastructure that may be required to implement this measure, but it is widely accepted that prevention is more cost effective than management of already introduced species (…). This measure would need to include the provision and training of administration and staff to enforce the regulations.	Medium
	Increasing public awareness, including education and training	Campaigns to educate people and increase awareness of invasive non-native species are an effective way to curb	Medium

	to reduce the risk of intentional (and un-intentional) introduction and spread: It is important to raise awareness about the possible consequences of introducing and spreading *A. nebulosus* to non-infested sites in the EU.	(il)legal introductions, especially those targeted at specific sectors e.g. anglers. Public awareness campaigns, however, do need to be maintained so they do not drop out of the collective consciousness, but are also renewed periodically to avoid fatigue. Ideally the development of awareness-raising campaigns and educational materials needs to be done for each member state, guided by scientific expertise and co-ordinated by an 'education committee' or a similar initiative. Resources required, and associated costs, are dependent upon the activities and materials developed, but may include media campaigns, websites, marketing materials, or outreach training and education schemes (Roy et al., 2018). Costs of campaigns to increase awareness are estimated to be low to medium (€50–200K/year) on an EU scale.	
Methods to achieve eradication [6]	**Effective surveillance and reporting:** *Ameiurus nebulosus* is a well-known species although confusion with its close congener, black bullhead *Ameiurus melas*, can be a problem. Effective eradication is most likely to be achieved when new invasions are quickly reported. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the species can become established.	Trawl nets, fyke nets, traps and electrofishing can be used to detect and monitor non-native fishes in the RA area, even if not always effective at low density (Britton et al., 2011) – in which case environmental DNA approaches can be used (e.g. Dougherty et al., 2016; Davison et al., 2017, 2019), including in large lakes (Larson et al., 2017). Environmental DNA-based monitoring could be considered also for the entire RA area. Citizen science could be promoted to monitor the possible introduction and spread of the species. If dedicated monitoring for *A. nebulosus* is not possible, then monitoring for this species can be incorporated in already running monitoring programmes (e.g. for the Water Framework Directive). Although the above mentioned tools may be effective in	Medium

	Post-eradication detection can also be undertaken to determine whether or not an eradication action has been successful. A simple and clear identification sheet could be drafted and distributed to different stakeholders (e.g. anglers, fishery managers) to increase the probability of an early detection and rapid response.	early detection, eradication of *A. nebulosus* after first find can only be effective when the detected infestation is low, with the potential feasibility and effectiveness of the eradication dependent on the size and type of infested water, with still waters being easier than water courses.Also, feasibility decreases (and costs increase) with increasing size of the water needing eradication action. In large riverine systems, which are a typical habitat of *A. nebulosus*, eradication may be impossible (see also below). The costs of dedicated surveillance and monitoring and subsequent removal of invasive fish are estimated to be medium (€200K–1M for five years) for the RA area. Eradication costs per surface area have been estimated in the U.K. as on average £20K GBP per hectare (≈ €22k/ha) (Britton et al., 2008).	
	Depletion and/or drain down of small standing waters: *A. nebulosus* is most commonly associated with stillwater environments, but it does occur in water courses.	Drain-down can be efficient and cost-effective, but is only feasible in some types of water bodies (e.g. fish ponds, small river reservoirs) and can be very destructive when rare or valuable fish species and other aquatic biota are negatively affected (e.g. Britton et al., 2010; Davison et al., 2019). Therefore, preferably, most fishes are mechanically removed prior to draining. The following methods may be suitable for depletion sampling and removal of fishes in the EU: electrofishing, seine nets, minnow traps, and fyke nets. All of these except electrofishing are more commonly used in still waters but can be used in water courses also. The likelihood of successful eradication, however, is low except in very small closed waters. The likelihood of success will decrease as stream size, stream discharge rate and water	Medium

		velocities increase. Although this type of measure can be successful in eradicating *A. nebulosus* populations, it is more designated to use as a management method. Cost are likely to be medium to high (>€50k/ha)	
	Use of piscicide (chemical removal): a piscicide can be used to kill newly-detected populations in smaller areas such as ponds, drainable larger water bodies (e.g. reservoirs), or small water courses. <u>There may be legal constraints as e.g. Rotenone was withdrawn from use in the European Union in 2007 (Schapira, 2010)</u>, but is still used in the U.K. (Britton et al., 2008, 2010; UK Environment Agency, 2014).	*Ameiurus nebulosus* can be killed by rotenone or other piscicides. However, it would be difficult (if not impossible) to make an effective eradication in large rivers and lakes. Use of rotenone was already successfully used for eradicating the invasive topmouth gudgeon *Pseudorasbora parva* in the U.K. (Britton et al., 2008, 2010) as well as the close congener of *A. nebulosus*, i.e. *A. melas*, from a small, isolated pond in Essex, England (UK Environment Agency, 2014). Use of rotenone is widespread in the U.S.A. (Ling, 2002), and has also been successfully applied in South Africa to eradicate the invasive smallmouth bass *Micropterus dolomieu* in a 4 km reach of the Rondegat River (Weyl et al., 2013, 2014). The potential to eradicate or control *A. nebulosus* populations depends on dispersal location and the level of establishment. If broadly dispersed in large lakes or river systems, eradication or control would likely be impossible. The costs of applying rotenone are high and were calculated for six ponds and lakes in the U.K. (for the eradication of topmouth gudgeon). The costs range from £6,600 (€7,500) for a small pond to £61,000 (€70,000) for a larger lake (Britton et al., 2010).	Medium
Methods to achieve	**Raising awareness:** Raising public awareness of the risks	Costs for outreach and production of leaflets can be high when applied across a large community, such as for the EU.	Low

management [7]	posed by IAS in general and *A. nebulosus* in particular will diminish the chance of new introductions after eradication/management of an invasive species. The production of targeted publicity and identification material is needed.		
	Mechanical removal: Mechanical removal of *A. nebulosus* can be done by fyke and seine netting, and electrofishing. Protocols for removal are well developed for a wide variety of fishes (West et al., 2007). Electrofishing is preferred because it has the least amount of by-catch and damage to native fish populations (Mueller, 2005). Angling can also be locally effective in removing large numbers of some invasive fishes (Savior, 2016).	Mechanical removal may be the only way to treat a system where chemical piscicides cannot be applied. Fyke and/or hoop nets have been effective in removing large numbers of *Ameiurus* catfishes and are a standard method to collect this species in many jurisdictions, e.g. New Zealand (Barnes & Hicks, 2003), North America (Miranda & Boxrucker, 2009; Pope et al., 2009) and Europe (Louette & Declerck, 2006; Cucherousset et al., 2006). A combination of fyke or hoop nets and electrofishing is considered a good form of mechanical removal for ictalurid catfishes (Prott et al., 2006; Miranda & Boxrucker, 2009). Double fyke nets, consisting of two conically shaped fyke nets (mesh size of 8 mm) of which the mouth openings are connected with a vertically hanging net (length, 11 m; height, 0.9 m), have been used effectively in Belgium (Louette & Declerck, 2006). Repeated removal attempts will result in higher efficiency. Angling and increased fishing effort by amateurs could also be part of the overfishing effort (Savior, 2016). Finally, the possibility of combing mechanical removal with drastic habitat alteration may also help or increase the synergistic pressures on an isolated population of invasive fishes such	High

		as these. *Ameiurus nebulosus* is difficult and costly to control (CABI, 2015). Data on detailed specifics and costs are generally lacking and are case specific. For example, Britton et al. (2010) estimated the cost for a near eradication of topmouth gudgeon (15 specimens left after five biomanipulation exercises) in a small (0.3 ha) and shallow (<1.5 m) lake to be £2,100. Investing in electrofishing equipment and fyke nets is needed. Additionally, gill nets and seine nets can be used. Prices of electrofishing gear are estimated to range from €750 to €4000 and more; fyke nets are between €500 and €1500, while gill nets are cheaper, but different mesh sizes need to be applied and high mortalities to non-target fishes may occur. The biggest costs are for operating these fishing gears and are an important extra cost to consider, as they are very labour intensive.	
	The **above methods** described to support eradication can also be used to manage existing *A. nebulosus* populations.	See above.	See above
	Reducing risks of further dispersal	Dedicated monitoring (e.g. electrofishing, fyke nets, trawl nets, but also eDNA) of water courses and water bodies is necessary to detect the presence of *A. nebulosus* and to ensure that these waters are not re-colonised by the species after eradication. In parallel, to prevent further spread and new introductions, a prohibition on the keeping and release should be enforced. Also, stringent procedures should be	Medium

		put in place to check within-EU consignments of fish intended for angling. Depending on the area that has to be monitored, management costs can be from medium to very high (from <€5k to > €1M).	
	Further research	Additional research on *A. nebulosus* is needed within the EU to understand better the risks posed by the species, with an initial step being a comprehensive literature review and meta-analysis of available biological traits, such as has been done for its close congener, *A. melas* (i.e. Copp et al., 2016). More detailed information is needed on the extent of the species distribution, which remains less well known than some other non-native species in Europe, e.g. pumpkinseed *Lepomis gibbosus* (Copp & Fox, 2007).	Medium

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: *Wasmannia auropunctata*, Roger 1863

Author(s) of the assessment:
Olivier Blight, Avignon university, Aix Marseille university, CNRS, IRD, IMBE, Avignon, France.

Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: Richard Shaw
Peer review 2: Marc Kenis
Peer review 3: Alan Stewart

Date of completion: 21/10/2019

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:
Class: Insecta
Order: Hymenoptera
Family: Formicidae
Genus: *Wasmannia*

Scientific name: *Wasmannia auropunctata* (Roger 1863)
Original name: *Tetramorium auropunctatum* (Roger 1863) (not valid)

Synonym names: *Wasmannia australis*, *Wasmannia laevifrons*, *Wasmannia nigricans*, *Wasmannia obscura*, *Wasmannia pulla*, *Wasmannia rugosa*: Longino & Fernández, 2007. A comprehensive and regularly updated list can be found at www.antweb.org, (Bolton 2019).

Common name: Little Fire ant (Wetterer and Porter 2003).

Also known as little red fire ant, little introduced fire ant, small fire ant, West Indian stinging ant, cocoa-tree ant (English); fourmi rouge, petite fourmi de feu, fourmi électrique (French, French-New Caledonia); fourmi Sangundagenta, tsanagonawenda (Gabon). A comprehensive list of local names is provided by Wetterer and Porter (2003).

Although *W. auropunctata* is variable, there is no evidence that it is composed of multiple cryptic species (Longino and Fernández 2007). *Wasmannia auropunctata* is less than 2 millimetres in length, orange/brown in colour, and very slow-moving and sluggish. It has long, pointy spines on the propodeum, two nodes (petiole and postpetiole), and two grooves on the front of the head where the antennae can lay at rest (antennal scapes) (Cuezzo et al 2015). There is a marked negative relationship between queen size and worker size in *W. auropunctata*.

A key for separation of the taxa in the *genus Wasmannia* was provided by Longino & Fernández (2007) and by Cuezzo et al. (2015).
Because other species of *Wasmannia* are rare and inconspicuous, this assessment covers only one species, *Wasmannia auropunctata*, Roger 1863.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

In practice, species from the genus *Wasmannia* may be confused with some species of the genus *Ochetomyrmex*. However, *Ochetomyrmex* have less developed antennal scrobes, the clypeal apron is lacking, and there is a slightly impressed mesonotal suture which is never present in *Wasmannia*. Species of *Ochetomyrmex* are all native from South America and are not known to be invasive nor are they recorded from Europe.

In Europe, *W. auropunctata* may be confused with species of *Solenopsis* (e.g. *Solenopsis fugax*) that are similar in size, colour and belong to the same sub-family (*Myrmicinae*). However, they have completely different ecology and behaviour. Species of the genus *Solenopsis* native from Europe are cryptic, forming small colonies that live under rocks or in the litter are almost never detectable on the soil surface. In contrast, *W. auropunctata* is not cryptic in its habits, it harbours several invasive traits, among which are behavioural and numerical dominance.
Invasive *Solenopsis* species, such as *Solenopsis invicta, S. richteri* and *S. geminata* (see previous risk assessments 2018) are much bigger and cannot be confused with *W. auropunctata*.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

A risk assessment has been carried out for New Zealand, which predicts that *W. auropunctata* would be unlikely (low risk) to establish outside but may achieve limited distribution in heated buildings (Harris et al 2005). It is predicted to have a low risk of spread from a site of establishment but the negative consequences of its presence are considered to be medium/high. The overall risk for New Zealand was considered to be low-medium. However due to the limited overlap in climatic and ecological conditions between New Zealand and the target area, this assessment has a limited relevance.

No other proper risk assessments have been carried for *W. auropunctata*. Several managing reports that are considered in the management annex are available (see for example Raymundo and Miller 2012; Vanderwoude et al. 2016).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:
W. auropunctata is native to Central and South America. It is often very common in Neotropical lowland forests (Wetterer 2013; www.antmaps.org; Guénard et al. 2017). *Wasmannia auropunctata* has been described as a true generalist in its choice of nest sites and habitats, allowing it to thrive in a wide range of conditions (Chifflet et al. 2018). The species is remarkably generalist in its habitat preference, it has invaded both open disturbed habitat and closed preserved forest in New-Caledonia (Berman et al. 2013a). It is common in habitats ranging from wet to dry and from early successional to mature.
The common ancestor of the two man clades occurred in central Brazil during the Pliocene (Chifflet et al. 2016). Clade A is present northward and clade B southward of Brazil. There are differences in the most suitable climate among clades, clade A being a tropical lineage and clade B a subtropical and temperate lineage. Only Clade B reached more southern latitudes, with a colder climate than that northern South America. This differentiation in climate suitability allowed this originally tropical ant to invade temperate climates.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

Wasmannia auropunctata has been extraordinarily successful in spreading into several continents (Africa, North America, South America, Europe, Australia) and has colonized many tropical islands.

In the New world *Wasmannia auropunctata* has spread throughout the West Indies and peninsular Florida. Because its known distribution from South America through the Lesser and Greater Antilles to Florida has no large gaps, it is not possible to determine where in the West Indies *W. auropunctata* is native and where it is exotic, and it seems likely that many islands have a mix of native and exotic populations (Wetterer 2013). Indoor records of *W. auropunctata* from temperate North America are certainly exotic. It has invaded many Caribbean islands and the Galapagos islands. From time to time it is occasionally detected in heated locales in Canada (Wetterer and Porter 2003).

In the Old world, populations of *W. auropunctata* have been documented in Gabon and neighbouring countries of Cameroon and The Central Africa Republic. In the Indo-Pacific, the earliest records date from 1972 in New Caledonia and 1974 on the Solomon Islands. These populations appear to be actively spreading, with recent records from Papua New Guinea and Guam. It is now spreading in many Pacific islands such as Hawaii and French Polynesia (See Wetterer (2013) for a detailed distribution and www.antmap.org).

In the Mediterranean basin, *W. auropunctata* has been recorded in Israel (first detection in 2005) and more recently in southern Spain (Espadaler et al. 2018). Polygyny and sexual production were ascertained. The infested area, in a suburb of Marbella (Málaga) is 5.8 ha in extent and 1.2 km perimeter.

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a):
Terrestrial biogeographic regions: Atlantic and Mediterranean.

Response (6b):
Terrestrial biogeographic regions: Mediterranean.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)

- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a):

- Mediterranean, Atlantic, according to Bertelsmeier et al. (2015a) (climatic suitability index[2] above 0.5, see annex I)

- Mediterranean, Atlantic, Continental, according to Federman et al. (2013) (climatic suitability index above 1, see annex II)

- Mediterranean, Atlantic, Continental, according to Coulin et al. (2019) (climatic suitability index above 0.5, see annex III). This model was built for the Mediterranean basin, and therefore does not cover the whole risk assessment area.

- Mediterranean and Atlantic according to Beckmann et al. (2019) (climatic suitability index above 0.5, see annex IV).

Response (7b):

- Mediterranean, Atlantic in 2080, according to Bertelsmeier et al. (2015a) (climatic suitability index above 0.5, prediction for 2080, see annex I)

- Mediterranean, Atlantic, Black Sea in 2070, according to Beckmann et al. (2019) (climatic suitability index above 0.5, see annex IV).

Bertelsmeier et al. (2015a), using a climate matching model (Maxent) based on present distributions, mapped suitable areas globally for *W. auropunctata*. To consider a range of possible future climates, they used downscaled climate data from three GCMs: the CCCMA-GCM2 model; the CSIRO MK2 model; and the HCCPR-HADCM3 model (GIEC 2007). Similarly, they used the two extreme SRES: the optimistic B2a; and pessimistic A2a scenario.

To estimate the effect of climate change on the potential distribution, Beckmann et al. (2019) computed equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-

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[2] A threshold rule was applied whereby all pixels with a probability of presence exceeding 0.5 were classified as "suitable" area. By convention, this threshold is frequently used for binary classification for species distribution modelling (See Bertelseier et al. 2015a).

LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip55m). Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats, we used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, night time lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality. Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

Federman et al. (2013) used the Maxent model to predict potential invasion and establishment of *W. auropunctata*. Bioclimatic variables were obtained from the WorldClim dataset. These variables were derived from the monthly temperature and rainfall values, in order to generate biologically meaningful variables. The bioclimatic variables represent annual trends, seasonality, and extreme or limiting environmental factors. Yearly reference evapo-transpiration was obtained from the database of the Food and Agriculture Organization of the United Nations (FAO).

Coulin et al. (2019) analysed 19 bioclimatic variables related to temperature and precipitation at 30 arc-seconds resolution available from WorldClim. After variables selection, the remaining variables were analysed with *W. auropunctata* clade B native range presence data to fit a SDM using the Maxent procedure. To explore the link between the thermo-physiological constraints and the SDM, the lower CTmin measured in their study was evaluated by analysing the latitudinal change of the minimum temperature of the coldest month (Bio6) and its effect on the probability of presence.

A number of underlying assumptions and inherent uncertainties are associated with the niche modelling approach and the actual distribution is contingent on many factors. This species distribution model is only based on climate data developed at a coarse scale. It does not include information on biotic interactions or other abiotic factors having an influence at a regional or global scale.

The choice of the 0.5 threshold is arbitrary. There is uncertainty about the potential and future geographic distribution of the species. Confidence will be increased with other SDMs.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):
W. auropunctata has been recently recorded in southern Spain, in the region of Malaga (Marbella) (Espadaler et al. 2018). Before that, it was first recorded in greenhouses in 1927 in United Kingdom, in 1988 in the Netherlands during import inspection at the Plant Protection Service (Boer and Vierbergen 2008) and in 2006 in Italy (Wetterer and Porter 2003).

Response (8b):
To date *W. auropunctata* is known to have established populations only in southern Spain, in the region of Malaga (Marbella) (Espadaler et al. 2018). The ants were first detected by local residents around 2016 but were probably introduced more than five years previously (Espadaler et al. 2018). The origin of this invasion is unknown and requires investigation.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a):

- France, Spain, Ireland, England, Italy, Greece and Croatia, according to Bertelsmeier et al. (2015a) (climatic suitability index above 0.5, see annex I).

- Austria, Germany, Hungary, France, Spain, Ireland, United Kingdom, Croatia, Greece, Sweden, Slovakia, Slovenia, Czech Republic, according to Federman et al. (2013) (climatic suitability index above 1, see annex II)

- France, Spain, Portugal, Italy, Slovenia and Croatia, according to Coulin et al. (2019) (climatic suitability index above 0.5, see annex 3). This model was built for the Mediterranean basin, and therefore does not cover the whole risk assessment area.

- Cyprus, France, Greece, Italy, Portugal, Spain, according to Beckmann et al. (2019) (climatic suitability index above 0.5, see annex IV).

Response (9b):

- France, Spain, Ireland, United Kingdom, Italy and Greece in 2080, according to Bertelsmeier et al. (2015a) (climatic suitability index above 0.5, see annex I).

- Croatia, Cyprus, France, Greece, Italy, Portugal and Spain in 2070, according to Beckmann et al. (2019) (climatic suitability index above 0.5, see annex IV).

Bertelsmeier et al. (2015a), using a climate matching model (Maxent) based on present distributions, mapped suitable areas globally for *W. auropunctata*. To consider a range of possible future climates, they used downscaled climate data from three GCMs: the CCCMA-GCM2 model; the CSIRO MK2 model; and the HCCPR-HADCM3 model (GIEC 2007). Similarly, they used the two extreme SRES: the optimistic B2a; and pessimistic A2a scenario.

A number of underlying assumptions and inherent uncertainties are associated with the niche modelling approach and the actual distribution is contingent on many factors. This species distribution model is only based on climate data developed at a coarse scale. It does not include information on biotic interactions or other abiotic factors having an influence at a regional or global scale.

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org). Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats, we used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, night-time lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality. Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

Federman et al. (2013) used the Maxent model to predict potential invasion and establishment of *W. auropunctata*. Bioclimatic variables were obtained from the WorldClim dataset. These variables were derived from the monthly temperature and rainfall values, in order to generate biologically meaningful variables. The bioclimatic variables represent annual trends, seasonality, and extreme or limiting environmental factors. Yearly reference evapo-transpiration was obtained from the database of the Food and Agriculture Organization of the United Nations (FAO).

Coulin et al. (2019) analysed 19 bioclimatic variables related to temperature and precipitation at 30 arc-seconds resolution available from WorldClim. After variables selection, the remaining variables were analysed with *W. auropunctata* clade B native range presence data to fit a SDM using the Maxent procedure. To explore the link between the thermo-physiological constraints and the SDM, the lower CTmin measured in their study was evaluated by analysing the latitudinal change of the minimum temperature of the coldest month (Bio6) and its effect on the probability of presence.

The choice of the 0.5 threshold is arbitrary. There is therefore uncertainty about the potential and future geographic distribution of the species. Confidence could be increased with improved SDMs that integrate for example physiological, trade routes or land use data.

Irrigation may be an important parameter that may facilitate *W. auropunctata* spread in the risk assessment area (Federman et al. 2013a).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:
Yes. It is considered to be amongst the most widely distributed invasive species on earth. It has colonized almost all continents and has ecological, economic, and health impacts (Holway et al. 2002). It is considered as one of the worst invasive ant species. It is present on the list of the 100 the world's worst invasive species of the IUCN as well as four other ant species (Lowe et al 2004).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:
Terrestrial biogeographic region: Mediterranean (Espadaler et al. 2018).

See reply to A12.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response: Spain

Espadaler et al. 2018 have described an infested area in Spain that has a perimeter of 1.2 km and 5.8 ha of surface. Although a number of other ant species are found around this infested area, none of these are detected within the infested area.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response:
There are no known socio-economic benefits of the species.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: "No information has been found."
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as "corridor" or "unaided)".
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[3] and the provided key to pathways[4].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

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[3] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[4] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9

Pathway name:

a) Transport-Stowaway (Hitchhikers in or on airplane). We also considered arrivals by any vehicular means from invaded areas outside the PRA area, whose journey time would not exceed a few days.
b) Transport-Contaminant (nursery material and other matters from horticultural trade)
c) Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)
d) Transportation of habitat material (soil, vegetation, wood, …)
e) Food contaminant (including live food)

Wasmannia auropunctata is considered one of the classic tramp ant species, due to its reliance on human-mediated dispersal and close association with humans (Hölldobler and Wilson 1990). It can hitchhike with many commodities through many pathways. However, only the entry of queen ants and nests present a risk of establishment.

This species reproduces mostly or entirely by nest budding rather than nuptial flights, and its natural long-range dispersal is limited (Lubin 1984). Therefore, *W. auropunctata* spreads in its non-native range primarily through human activities (Holway et al. 2002), such as transfer of plants, soil, food packaging, logs, and wood products (Lubin 1984; Roque-Albelo and Causton 1999; Wetterer and Porter 2003; Wetterer 2013).

Pathways for the introduction of *W. auropunctata* to new locations include both natural (e.g., floating on vegetation and debris), and human-mediated routes (e.g., the nursery trade, transportation in soil, packaging materials moved by road sea or air). *Wasmannia auropunctata* has been intercepted from a variety of commodities (ornamental plants and fruits) and origins (South America, Pacific islands) at US ports and airports since 1910 (Blight et al. unpublished data). One hypothesis for the introduction of *W. auropunctata* in Hawaii is transportation along with fish-tail palms. Causton et al. (2005) suggested that it is easily transported on fruits and vegetables and that the growing trade between countries has facilitated the spread of *W. auropunctata*.

Harris et al (2005) provided a very detailed analysis of potential pathways of introduction of *W. auropunctata* in New Zealand, which is also relevant for Europe.

Queens may enter the risk assessment area through the ant market on the internet. This pathway should be considered in the future if the online market of ants is not sufficiently regulated.

a) Transport-Stowaway (Hitchhikers in or on passenger aircraft). We also considered arrivals by any vehicular means from invaded areas outside the PRA area whose journey time would not exceed a few days.

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns only newly mated queens. Indeed, it is very unlikely that established nests will travel in or on a passenger aircraft without being transported in containers or nursery materials. In contrast, queens during the nuptial flight periods can accidently enter into a passenger aircraft.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response:

Wasmannia auropunctata has a casual association as a hitchhiker/stowaway with freight and in particular air passengers. For example, of 11 interceptions in Australia, most were from air passengers (mostly carrying plants, cut-flowers, or woven baskets or matting) (data from January 1986 to 30 June 2003; Department of Agriculture, Fisheries and Forestry, Canberra cited in Harris et al 2005).

Air passengers from South and central America countries with known infestations represent one of the most likely pathways to Europe (Foucaud et al. 2010a). Many individuals may travel this pathway. Although little data is available on ant interceptions at ports and airports, the proportion of queens recorded in these interception databases is very low. This suggests a relatively low number of newly-mated queens travelling along this pathway.

Newly emerged queens and males have wings and Torres et al. (2001) collected large numbers of *W. auropunctata* in light traps on Puerto Rico. However, such flights of males and females seem to occur only in the native range of *W. auropunctata*. Independent nest founding is considered highly unlikely (Ulloa-Chacon 1990, cited in Harris et al 2005). Colonies mainly reproduce through budding by which a group of workers leave the nest with a queen to start a new colony within a few metres, in both the invaded areas and in native urban areas (Chifflet et al. 2018). This limits the success of a lone queen in colony foundation. Indeed, in this type of reproduction, the likelihood of unaided queens starting a new colony is very low (Hölldobler and Wilson 1977).

No data is available to estimate the role of propagule pressure on the likelihood of introduction, but because *W. auropunctata* mainly reproduces through budding, propagule pressure may be low. Indeed, under this scenario that only considers the transport of new queens, propagules might concern only the species native range. Dependent colony foundation involves a queen and several workers that are unlikely to reach an aircraft without being transported in containers or nursery materials.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
In their native range where queens have independent colony foundation, the queens are likely to be able to survive several tens of days using their own reserves before the first workers emerge. However this means of colony formation in the invaded range seems to be rare in *W. auropunctata* (Causton et al. 2005). Reserves decrease in queens that need workers to start new a colony (Hölldobler and Wilson 1990). The likelihood of survival will thus decrease with increasing travel duration, but survival is possible. Multiplication and the establishment of a small nest during such an intercontinental flight however is highly unlikely.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
N/A. There are no management practices in place against hitchhiking ants or ant queens in or on airplanes.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Importation via this pathway is not likely to be detected by current surveillance. Detection rates for solitary queens or even several queens are low; in general, ants are not easy to detect in cargo airplanes and detection rate thus will be low. This is particularly true for tiny ants such as *W. auropunctata* whose workers measure around 1.5 mm and queens less than 3 mm.

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response:
The likelihood is scored moderately likely because the number of queen ants travelling through this pathway is expected to be relatively low because it may concern only the native range of the species and the duration of the transportation would not favour the survival of the queen.

b) Transport-Contaminant (nursery material and other material from the horticultural trade)

Qu. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns both small groups of workers and a queen dispersing through budding, and fully developed nests (with active workers) transported in nursery material by the horticultural trade.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:

There are very limited data on ant nests arriving through the horticultural trade in Europe. At least some nests have reached Europe (Spain) and Israel in the Mediterranean basin also. In the Netherlands, *W. auropunctata* was intercepted between two to five times during import inspections at the Plant Protection Service (Boer & Vierbergen 2008).

Ants are not listed as quarantine pests in the EU and, therefore, records rarely appear in the national and international lists of intercepted pests. However, millions of plants arrive in Europe with soil or in pots (with substrates) from infested areas (South America, Central America, Southern US, Caribbean islands, Pacific islands and south Asia) every year and, although the soil/substrate is supposed to be sterile, infestation by ants can occur just before or during transport. The European Union (EU) imports a large volume and diversity of plants for planting every year, and the value of imported plants for planting has increased 60% over the past fifteen years (Eschen et al. 2015). For example, in the period 2013-2017, the annual volume of EU imports from the US of live plants (CN code 0602) varied between 3,000 and 5,200 tonnes with value between 11 and 16 million euro. The US was the fifth largest exporter to the EU of these products in volume and number eight in value. The US share of the total EU imports of live plants was 1% in volume and 4% in value.

Flower pots are one of the preferred habitats for invasive ants in invaded regions, in particular because of their humidity and because they are usually in contact with the ground. Other horticultural material such as mulch, hay and other plant material can also harbour ant nests.

Both multiple queened (polygyne) and single queened (monogyne) colonies occur (Wetterer & Porter 2003). *Wasmannia auropunctata* reproduces through clonal or sexual reproduction (Foucaud et al. 2009) and forms only polygynous colonies in its introduced areas or in native urban areas (Chifflet et al. 2018). The number of workers in a polygynous nest can reach around 5000 workers/m^2 in areas where it is abundant (Clark et al. 2006). Single nests of *W. auropunctata* may contain several mated queens, numerous workers, pupae, larvae and eggs. Nest densities are higher in areas where this species has become a pest in its introduced range (0.75-2.7 aggregations/ m^2 on the Galápagos) (Lubin 1984). Ant nests might travel on the pathway in large numbers as a contaminant of horticultural materials containing soil.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Groups of active workers with queens are able to survive a few weeks with no food. In the case they do not find food resources they can eat their eggs and larvae. Moreover, some ant species (e.g. *Temnothorax rugatulus*) can survive for several months without food resources (Rueppell and Kirkman 2005).
However, because *W. auropunctata* has a generalist diet, they are likely to find food during the transport. Tropical ants like *W. auropunctata* require moisture for their survival. However, it is unlikely to be a limiting factor along this pathway.

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:

Horticulture plants and soils/substrates are often chemically treated before shipment but there are no known existing management practices during transport and storage under current regulations. Horticultural plants and soils/substrates can be infested after treatment either before departure or during transport. There is little information available on management during transport or its efficacy.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Fully developed nests can be detected despite the workers being tiny and similar in colour to many soils. However, a newly-founded colony of a queen(s) and workers in the soil/substrate can easily arrive undetected.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Given the high numbers of horticulture items imported into Europe each year from infested areas, the probability of introduction along this pathway is likely. Since 1920 more than 60% (45 out of 76 interceptions) of the interceptions at ports and airports in US were from nursery material and other matters from horticultural trade (mostly *Vitis* plants and orchids) (Blight et al unpublished data). *Wasmannia auropunctata* is most likely to have been transported between the large islands in the Galapagos archipelago on plants and in soil (Roque-Albelo and Causton, 1999).

Conversely, interceptions (11 interceptions) in Australia were mainly from air passengers (mostly carrying plants, cut-flowers, or woven baskets or matting) (data from January 1986 to 30 June 2003; Department of Agriculture, Fisheries and Forestry, Canberra cited in Harris et al. 2005).

c) Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)

Qu. 1.2c. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This section includes travelling nests that are not directly associated with the horticultural trade. Virtually any article of commerce can host hitchhiking nests of all sizes and ages, including newly-founded colonies and fully developed colonies. A free volume of 10ml should be

sufficient for an incipient colony composed by a queen and a dozen of workers. There are very many articles of commerce and container types that are grouped together here. This includes, e.g. sea containers but also vehicles (incl. used car parts), machinery, building material, packaging materials, bark, aquaculture material and used electrical equipment.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:

There are very limited data on ant nests arriving in Europe. Sea containers and all articles of commerce cited above were considered by Harris et al. (2005) as the main source of transport for *W. auropunctata*. Ant nests might travel along the pathway in large numbers as stowaways in containers or other bulk freight, including soil, fruits and vegetable. However, as presented above (Q1.3b), polygynous nests can reach high densities (5000 workers/m^2 and several queens) which increases the chances of a large number of nests (group composed of workers and one or several queens) to be transported from one invaded area.

The movement of large numbers of workers increases colony survival. However, it is of less concern compared to mated queens as workers do not reproduce.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
The chances of queens surviving transport along this pathway is very likely as workers will feed them. The likelihood of nest survival along this pathway is high. In the case that they do not find food resources they can eat their eggs and larvae. Moreover, some ant species (e.g. *Temnothorax rugatulus*) can survive for several months without food resources (Rueppell and Kirkman 2005).

However, though the likelihood of survival is high, this will decrease with increasing travel duration. Multiplication of a small nest during intercontinental translocation however is probably unlikely and will depend on the availability of resources.

Qu. 1.5c. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
For most of the commodities in this pathway, there are no management practices in place.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Many of these commodities are not carefully inspected. While established nests are usually obvious, small nests are often inconspicuous. Newly-founded nests with a queen and workers could easily arrive undetected. The tiny size of both queens and workers makes the detection of this species difficult. A free volume of 10ml should be sufficient for an incipient colony composed of a queen and a dozen of workers, making their detection almost impossible.

Qu. 1.7c. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Given the high numbers and multiple types of containers, commodities and items that can be associated with *W. auropunctata*, this pathway can be considered as having a high likelihood of entry, as determined by Harris et al. (2005). Since 1920 around 40% of the interceptions at ports and airports in US were from Yam tubers, ginger, corn but also wood pallet or crate (Blight et al unpublished data).
It is likely that *W. auropunctata* was transported between small Galapagos islands on camping provisions and equipment (Roque-Albelo and Causton 1999).

d) Transportation of habitat material (soil, vegetation, wood, …)

Qu. 1.2d. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:

This concerns both small groups of workers and a queen dispersing through budding, and fully developed nests (with active workers) transported in soil or vegetation.

Qu. 1.3d. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:

There are very limited data on ant nests arriving in Europe. At least some nests have reached Europe (Spain) and Israel in the Mediterranean basin also. In the Netherlands, *W. auropunctata* was intercepted between two to five times during import inspections at the Plant Protection Service (Boer & Vierbergen 2008).

Nests are likely to be transported if the soil or vegetation of an infested sites are moved into Europe. However, the volume of such trade remains unknown, and the likelihood of the introduction of infested habitat from overseas is probably very low.

Both multiple queened (polygyne) and single queened (monogyne) colonies occur (Wetterer & Porter 2003). *Wasmannia auropunctata* reproduces through clonal or sexual reproduction (Foucaud et al. 2009) and forms only polygynous colonies in its introduced areas or in native urban areas (Chifflet et al. 2018). The number of workers in a polygynous nest can reach around 5000 workers/m^2 in areas where it is abundant (Clark et al. 2006). Single nests of *W. auropunctata* may contain several mated queens, numerous workers, pupae, larvae and eggs. Nest densities are higher in areas where this species has become a pest in its introduced range (0.75-2.7 aggregations/ m^2 on the Galápagos) (Lubin 1984). Ant nests might travel on the pathway in large numbers as a contaminant of horticultural materials containing soil.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:

Groups of active workers with queens are able to survive a few weeks with no food. If they do not find food resources they can eat their eggs and larvae. In fact, some ant species (e.g. *Temnothorax rugatulus*) can survive for several months without food resources (Rueppell and Kirkman 2005).
However, because *W. auropunctata* has a generalist diet, they are likely to find food during the transport. Tropical ants like *W. auropunctata* require moisture for their survival. However, it is unlikely to be a limiting factor along this pathway.

Qu. 1.5d. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
There is no information available on management during transport or its efficacy along this pathway.

Qu. 1.6d. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**Very likely**	**CONFIDENCE**	**medium**

Response:
The probability of detection will be negatively correlated to the volume of soil of vegetation transported. Fully developed nests might be detected despite the workers being tiny and similar in colour to many soils. A newly-founded colony of a queen(s) and workers in the soil/substrate could easily arrive undetected.

Qu. 1.7d. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Wasmannia auropunctata could be transported effectively along this pathway as the transfer of soil of vegetation are suitable habitat for the species survival. However, the propagule pressure is unknown, the probability of habitat material transfer from both the native and introduced ranges into Europe might be low outside of the plants for planting pathway that includes habitat.

e) Food contaminant (including of live food)

Qu. 1.2e. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns both small groups of workers and a queen dispersing through budding, and newly-mated queens transported with fruits or vegetables.

Qu. 1.3e. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
There are very limited data on ant nests and queens arriving in Europe. At least some nests have reached Europe (Spain) and Israel in the Mediterranean basin also. In the Netherlands, *W. auropunctata* was intercepted between two to five times during import inspections at the Plant Protection Service (Boer & Vierbergen 2008).

Europe has a large and mature market for fresh fruits and vegetables with stable demand overall. The need for year-round availability and the interest in new exotic produce maintain Europe’s continuous dependence on external suppliers. With a population of more than 500 million consumers, Europe is responsible for 45% of the global trade value of fresh fruits and vegetables. Five of the global 10 importing countries are in Europe. The total import value from developing countries increased 38% in five years to 18.2 billion euros in 2018, which is significantly larger than the 3.1 billion euros in imports from developed, non-European countries, which grew by 20% in the same period (source www.cbi.eu). Some of the main countries that export fruits and vegetables to Europe are either in the native or introduced range of the species (e.g. Mexico, Peru, Brazil, Argentina, Costa Rica and Guatemala) (source www.cbi.eu).

Ants are not listed as quarantine pests in the EU and, therefore, records rarely appear in the national and international lists of intercepted pests. However, millions of tons of fruits and vegetables arrive in Europe from infested areas (South America, Central America, Southern US, Caribbean islands, Pacific islands and South Asia) every year and, although they are supposed to be washed, infestation by ants can occur just before or during transport. *Wasmannia auropunctata* has been intercepted at ports and airports in US on Yam tubers, *Zea mays* and *Zingiber officinale*. Ant interceptions on food represent 34% of the total records in US.

Both multiple queened (polygyne) and single queened (monogyne) colonies occur (Wetterer & Porter 2003). *Wasmannia auropunctata* reproduces through clonal or sexual reproduction (Foucaud et al. 2009) and forms only polygynous colonies in its introduced areas or in native urban areas (Chifflet et al. 2018). Small nests or newly-mated queens may travel on the pathway in large numbers as a contaminant of fruits and vegetables.

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 1.4e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
The probability of nest or solitary queens' survival is very likely along this pathway as ants are likely to find food resources. Tropical ants like *W. auropunctata* require moisture for their survival. However, this is unlikely to be a limiting factor along this pathway.

Wasmannia auropunctata has been intercepted at ports and airports in US on Yam tubers, *Zea mays* and *Zingiber officinale*. In general, ant interceptions on food represent 34% of the total interception records in the USA.

Qu. 1.5e. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Fruits and vegetables are often washed before shipment but there are no known existing management practices under current regulations during transport and storage. Fruits and vegetables can be infested after treatment either before departure or during transport. There is little information available on management during transport or its efficacy.

Qu. 1.6e. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
A newly-founded colony of a queen(s) and workers or a solitary queen can easily arrive undetected.

Qu. 1.7e. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Given the high numbers of food (fruits and vegetable) items imported into Europe each year from infested areas, the probability of introduction along this pathway is likely. Since 1920 more than 34% of the ant interceptions at ports and airports in US were from food (mostly *Zea mays* and *Zingiber officinale*) (Blight et al unpublished data).

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.
Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
The species has been already recorded/intercepted in Europe and it is likely that this will happen again, specifically with contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freight.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:

Climate change is not changing the risk of introduction or likelihood of entry based on the mentioned active pathways.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[5] and the provided key to pathways[6].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.
For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.
In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name:

a) Transport-Stowaway (Hitchhikers in or on passenger airplane) We also considered arrivals by any vehicular means from invaded areas outside the PRA area whose journey time would not exceed a few days.
b) Transport-Contaminant (nursery material and other matters from horticultural trade)
c) Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)
d) Transportation of habitat material (soil, vegetation, wood, …)
e) Food contaminant (including of live food)

See question 1.1 for details.

Qu. 2.2a. Transport-Stowaway (Hitchhikers in or on airplane). We also considered arrivals by any vehicular means from invaded areas outside the PRA area whose journey time would not exceed a few days.

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

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[5] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[6] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns only newly-mated queens without workers.

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response:
Most new colonies are established by queens aided by a group of workers (dependent colony foundation), which decreases the probability of entry of lone queens. However, the entry of queens that originated from areas where *W. auropunctata* reproduces by nuptial flight must be considered. This is the case in the native range and at least in the Galápagos Islands (Meier 1994 cited in Harris et al 2005) in the invaded range, although independent colony foundation was not demonstrated.

See Q1.3a for more details on species reproduction, propagule pressure, and the volume of movements along this pathway.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Considering the small size of *W. auropunctata* queens (< 3mm) and the queens’ hiding behaviour when attempting to start a new colony, their entry into the risk assessment area undetected is likely.

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	CONFIDENCE	high

Response:
Although *W. auropunctata* is a tropical species, studies demonstrated a shift in population thermotolerance in the native range (Orivel et al. 2009; Chifflet et al 2018). Populations can exist in habitats with very different climatic conditions. In the native range the annual temperature remains stable at values below 30°C and humidity never drops below 80% in natural habitats, whereas in invaded human-modified habitats, temperatures may reach 40°C and humidity may drop to 50%. This is confirmed by the establishment of an invasive population in Israel that has much harsher conditions (colder in winter, and warmer and drier in summer) (Vonshak et al. 2009) and by the recent southern expansion of native populations in Argentina (Rey et al. 2012; Chifflet et al. 2018). Workers start to forage at 6°C (Coulin et al. 2019), which increases its chances of entry during the most appropriate months of the year.

Sexuals are produced throughout most of the year (Passera 1994) and reproduction of ant queens can occur over several months and commodities along with which ants can be imported into Europe throughout the year.

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	likely	CONFIDENCE	medium

Response:
Many airports in the Mediterranean region are surrounded by suitable habitats including irrigated gardens and parks. Indeed, this species as an invasive ant simply requires soil as a substrate in which to establish a nest and has been found to occur in diverse degraded habitats with a wide range of climatic conditions (see section A4 for a more comprehensive description of the species habitat requirements). The recent invaded area in Spain is not different from other areas in the Mediterranean region, which supports the likelihood of queens' transfer to suitable habitats.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	likely	CONFIDENCE	medium

Response:
The likelihood is scored likely because the number of queens travelling through this pathway is expected to be relatively low and the duration of the transportation would not favour the survival of the queens.

Qu. 2.2b. Transport-Contaminant (nursery material and other matters from horticultural trade) **Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?**

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns both fully developed colonies (with many active workers) and newly-founded nests (nucleus of workers and a queen that left the nest to start a new colony) transported in nursery material for the horticultural trade. Newly-founded colonies can also be formed by queens transported in ships before the nursery material arrives at destination. However, independent colony foundation has never been observed in *W. auropunctata* despite observations of nuptial flights.

Whilst entry is almost always unintentional, *W. auropunctata* was intentionally introduced in cacao plantations in Cameroon to biologically control pest insects, particularly Hemiptera (Wetterer et al 1999).

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
There are very limited data on ant nests arriving through the horticultural trade in Europe. At least some nests have entered in Europe (Spain), New Zealand, Australia, US and several Caribbean and Pacific islands.
Considering this pathway as one of the main sources of introduction, it is likely that a large number of colonies will enter in the risk assessment area along this pathway. Millions of plants arrive with soil or in pots (with substrates) from infested areas (Southern US, Mexico, Caribbean islands and China) every year in Europe and, although the soil/substrate is supposed to be sterile, infestation by ants can occur just before or during transport.

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:

Fully developed nests can be detected despite the workers being tiny and light brown to golden brown, making them harder to detect in the soil. However, newly-founded colonies of few queen(s) and workers in the soil/substrate can easily enter undetected.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
The horticultural trade is active throughout the year and populations of *W. auropunctata* both in native and invaded areas show pre-adaptation to temperate climatic conditions (see Q2.5a).

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Potted plants and plant materials are likely to be transported outdoors in gardens, which may adjoin a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion. This is supported by the high propensity of *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Given the high numbers of horticulture items imported into Europe each year from infested areas, the probability of entry along this pathway is high. Since these ants have an affinity for nesting at tree bases and in potted plants, they are easily spread between plant nurseries. When contaminated plants are purchased and planted, the ants are likely to enter into the environment.

Qu. 2.2c. Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This section includes travelling colonies that are not directly associated with the horticultural trade. Virtually any article of commerce can host hitchhiking nests of all sizes and ages,

including newly-founded and fully developed colonies. This section considers a wide range of articles such as sea containers, vehicles (incl. used car parts), machinery, building material, packaging materials, bark, aquaculture material and used electrical equipment.

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
There are very limited data on ant nests entering in Europe. At least some nests have entered Europe (Spain), New Zealand, Australia, US and several Caribbean and Pacific islands. Sea containers and all articles of commerce cited in Q2.2c were scored by Harris et al. (2005) as presenting a high likelihood of introduction for nests.

Propagule pressure may be high since the number of incidents are likely to be high and the fact that the number of workers in a polygynous colony can reach around 5000 workers/m^2 in areas where it is abundant (Clark et al. 2006).

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Many of these commodities are not carefully inspected. While established nests may be obvious despite the workers being very small, newly-founded colonies are often inconspicuous. Newly-founded colonies with few queen(s) and workers could easily arrive undetected.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Commodities that can carry *W. auropunctata* are introduced to the risk assessment area throughout the year and populations from both native and invaded areas show pre-adaptation to temperate climatic conditions (see 2.5a).

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Several of the potential commodities and items in which nests can hide can be transported to suitable habitats since the ant particularly likes disturbed habitats, which are found everywhere, specifically in urban and semi-urban habitats. This is confirmed by the high propensity for *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Given the high numbers and types of containers, commodities and items that can be associated with *W. auropunctata*, this pathway can be considered as having a high likelihood of entry, as determined by Harris et al. (2005). However, contrary to the horticulture pathway, the final destination of some items (e.g. vegetables or fruits or electrical equipment) may decrease the likelihood of release in nature.

d) Qu. 2.2d. Transportation of habitat material (soil, vegetation, wood, …)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns both fully developed colonies (with many active workers) and newly-founded nests (nucleus of workers and a queen that have left the nest to start a new colony) transported in soil or vegetation during the movement of habitat material. Newly-founded colonies can also be formed by queens transported in ships before the shipment arrives at destination. However, independent colony foundation has never been observed in *W. auropunctata* despite observations of nuptial flights.

Whilst entry is almost always unintentional, *W. auropunctata* was intentionally introduced in cacao plantations in Cameroon to biologically control pest insects, particularly Hemiptera (Wetterer et al 1999).

Qu. 2.3d. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**unlikely**	**CONFIDENCE**	**low**

Response:
There are very limited data on ant nests arriving through the horticultural trade in Europe. At least some nests have entered in Europe (Spain), New Zealand, Australia, US and several Caribbean and Pacific islands.
Considering the low probability of habitat transfer from overseas into Europe, it is unlikely that a large number of colonies will enter in the risk assessment area along this pathway.

Qu. 2.4d. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
The probability of detection will be negatively correlated to the volume of soil of vegetation transported. Fully developed nests might be detected despite the workers being tiny and similar in colour to many soils. A newly-founded colony of a queen(s) and workers in the soil/substrate can easily enter undetected.

Qu. 2.5d. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:

Such trade may be active throughout the year and populations of *W. auropunctata* both in native and invaded areas show pre-adaptation to temperate climatic conditions (see Q2.5a).

Qu. 2.6d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Soil and vegetation are likely to be transported to gardens, which may adjoin a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion. This is supported by the high propensity of *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 2.7d. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Wasmannia auropunctata can be transported in large amount along this pathway as soil or vegetation are highly suitable habitats. However, the propagule pressure is unknown outside of the trade n plants for planting, the probability of the transfer of habitat (soil and vegetation) from both the native and introduced ranges into Europe along this pathway might be low.

e) Qu. 2.2e. Food contaminant (including of live food)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
This concerns both newly-founded nests (nucleus of workers and a queen that left the nest to start a new colony) and solitary queens transported with food (e.g. fruits and vegetables). Newly-founded colonies can be formed by queens transported in ships before the nursery material arrives at destination. However, independent colony foundation has never been observed in *W. auropunctata* despite observations of nuptial flights.

Qu. 2.3e. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.

- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:

There are very limited data on ant nests arriving in Europe. At least some nests have reached Europe (Spain) and Israel in the Mediterranean basin also. In the Netherlands, *W. auropunctata* was intercepted between two to five times during import inspections at the Plant Protection Service (Boer & Vierbergen 2008).

Ants are not listed as quarantine pests in the EU and, therefore, records rarely appear in the national and international lists of intercepted pests. However, millions of tons of fruits and vegetables arrive in Europe from infested areas (South America, Central America, Southern US, Caribbean islands, Pacific islands and South Asia) every year and, although the they are supposed to be washed, infestation by ants can occur just before or during transport. *Wasmannia auropunctata* has been intercepted at ports and airports in US on Yam tubers, *Zea mays* and *Zingiber officinale*. Interceptions on food sources represent 34% of the total records in US.

Qu. 2.4e. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Newly-founded nests may be detected despite the workers being tiny and light brown to golden brown, making them harder to detect in the soil. Newly-mated queens can easily enter undetected.

Qu. 2.5e. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
This trade is active throughout the year and populations of *W. auropunctata* both in native and invaded areas show pre-adaptation to temperate climatic conditions (see Q2.5a).

Qu. 2.6e. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
Food is likely to arrive in warehouses, which may adjoin a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion. This is supported by the high propensity of *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 2.7e. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Since these ants can nest in disturbed areas, they can easily find a suitable habitat nearby. When contaminated food is stored or purchased, the ants are likely to enter into the environment.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.
Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
The species has been already recorded/intercepted in Europe and it is likely that this will happen again, most likely via contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freights.

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.
Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:

Climate change will not change the risk of introduction or likelihood of entry based on the specified active pathways.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
It is likely that *W. auropunctata* will establish colonies in the risk assessment area. An established population was already recorded in southern Spain (Espadaler et al. 2018). The species is also present in Israel under harsher climatic conditions (Vonshak et al. 2009; Vonshak et al. 2010). Despite these contrasting abiotic conditions, the Israeli populations display nesting and foraging behaviour similar to that observed in tropical and subtropical areas (Vonshak et al. 2010). The population in Israel originates from a native population that had extended its distribution south in Argentina under a temperate climate (Rey et al. 2012). This population seems to be pre-adapted, which increases the likelihood of colony establishment in the risk assessment area. The origin of the European population in Spain has to be determined to confirm this hypothesis.

Bertelsmeier et al. (2015a), using a climate matching model (Maxent) based on present distributions, mapped suitable areas globally for 15 of the worst invasive ant species (incl. *W. auropunctata*) (Annex I). They showed that around 5% of the European continent is presently suitable for *W. auropunctata*. However, this model seems to be more conservative than the Maxent model developed by Federman et al. (2013) (Annex II). In this later model, irrigation was included as a variable. This corrected model predicted a larger suitable area in Europe, including the continental biogeographic region that is absent from Bertelsmeier et al (2015a). Beckmann et al. (2019) found that both the Mediterranean and the Atlantic regions are at risk of species establishment (Annex IV).

Urbanisation is another key factor that determines the success of invasive ants' establishment (Holway et al. 2002). *Wasmannia auropunctata* is highly competitive in such habitats (Orivel et al. 2009; Vonshak et al. 2010), and there is little doubt that it will find suitable urban areas, even under northern latitudes to establish colonies.

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	**moderately widespread**	**CONFIDENCE**	**high**

Response:
Wasmannia auropunctata prefers disturbed habitats, which are found everywhere in Europe. However, even though some populations are more thermotolerant (Rey et al. 2012), as a tropical species it needs elevated temperatures to complete its life cycle, which may limit its distribution to the Mediterranean and Atlantic regions, at least in natural areas. Climatic records show that it can survive in areas with minimum temperatures ranging from 8°C to 22.7°C and maximum temperatures ranging from 29°C to 39.7°C, as well as a maximum of 12 months with less than 15 mm precipitations (Vonshak et al. 2010). The critical thermal maximum for both workers and queens is around 42°C and their critical thermal minimum is around 3.7°C (Coulin et al. 2019).

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	**N/A**	**CONFIDENCE**	**high**

Response:
Wasmannia auropunctata does not require another species for establishment.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Wasmannia auropunctata is an ecologically successful dominant ant both in disturbed and protected ecosystems in areas to which it has been introduced. *Wasmannia auropunctata* appears to be highly competitive compared with other invasive ant species. *Wasmannia auropunctata* was ranked first during competitive confrontations with six other highly invasive ants under laboratory conditions (Bertelsmeier et al. 2016). This is confirmed by the massive impacts it has on other ants in nature (Jourdan 1997; Holway et al. 2002; Vonshak et al. 2010; Berman et al. 2013a).

In several suitable areas it will have to face the competition with two invasive species, the Argentine ant *Linepithema humile* and *Tapinoma magnum*. These species are highly competitive (Blight et al. 2010; Blight et al. 2014) and confrontations will be asymmetric as they both already form supercolonies of many hundred thousand individuals. However, *W. auropunctata* was superior to the Argentine ant under laboratory confrontations (Bertelsmeier et al. 2015b; Bertelsmeier et al. 2016). The Argentine ant is largely distributed along the Mediterranean coast from Portugal to Italy through Spain and France. Moreover, these competitor species have a more temperate distribution and may have a competitive advantage over *W. auropunctata* in those parts of the risk assessment area. Nonetheless, where these competitive species are not present, establishment may easily occur.

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
No species of the genus *Wasmannia* are native to Europe, no specialist natural enemies of *Wasmannia* are known to occur in Europe. Thus, establishment in Europe is only likely to be hindered by other ant species and possibly generalist predators that may prey on individual queens.

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
No specific management practices are in place against invasive ants in the wild in Europe. Eradication of single nests is straightforward in buildings but much less so outdoors. However, some eradication programmes have succeeded at a local scale, such as over 2ha on Santa Ge Island (Galapagos) (Hoffmann et al. 2016).

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	**very unlikely**	**CONFIDENCE**	**high**

Response:
There have been no management practices applied in the risk assessment area but conventional management practices to date should not facilitate establishment (Hoffmann et al. 2016).

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
The eradication of *W. auropunctata* outdoors is difficult, especially when populations reach high densities of nests and individuals within those nests. Only killing of the queens will eradicate the population, which requires the use of toxin with a delayed action to reach that queens that are protected inside the nest.
An invasive population has been successfully eradicated over 21 ha in the Galapagos Islands after a nine months period of treatments (Causton et al. 2005). This is the largest successful

eradication campaign. However incipient colonies can be successfully eradicated (Hoffmann et al. 2016)

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**Very likely**	**CONFIDENCE**	**high**

Response:
Mikheyev et al. (2008) showed that the probability of establishment increases with propagule pressure in *W. auropunctata* although others factors such as local biotic and abiotic conditions may determine establishment success.

Despite *W. auropunctata* normally having single queen (monogynous) populations in the native range, clonal polygynous forms are mainly found both in introduced areas and in native disturbed habitats. The polygynous form can more easily establish because the higher number of queens increases reproduction potential, especially in the critical early stages of establishment.

In polygynous populations, the density of nests is more than 100 times higher than in native natural habitats. The number of queens and workers in a polygynous nest can vary enormously, from 35 to 90 queens per m^2 and from 500 to 2,500 workers per m^2 in New Caledonia (Orivel et al. 2009). In the Galapagos Islands, Clark et al. (2006) estimated the number of workers at 5,000 individuals/m^2 in areas where it is abundant.

Sexuals are produced throughout most of the year (Passera 1994) and can reproduce under varying climatic conditions.

The division of labour, i.e. the existence of reproductive caste, enabled ants to become ecologically dominant invertebrates in terrestrial habitats, with a high success rate of reproduction and dispersal. For example in the case of the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient for a colony to grow quickly (Hee et al. 2000; Luque et al. 2013).

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	**Very likely**	**CONFIDENCE**	**high**

Response:
Wasmannia auropunctata is one of the most widespread invasive ants. Despite it being considered a tropical ant, its southern expansion in its native range and as well as its presence in Spain and Israel highlights its adaptability to various climatic conditions (Vonshak et al. 2009; Rey et al. 2012; Chifflet et al. 2018). This adaptability is evident from laboratory cold-tolerance tests which showed that workers from populations established in Israel survived significantly better and recovered faster than populations from northern part of its distribution (native and introduced areas) (Rey et al. 2012).

Wasmannia auropunctata favours environments that are associated with humans, but it can colonise both open and closed habitats (Orivel et al 2009; Chifflet et al. 2018). However, several factors can constrain establishment of this species. Humidity is required for the survival of the species and may be a key factor in defining suitable habitats (Federman et al. 2013).

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	**Very likely**	**CONFIDENCE**	**high**

Response:
Most invasive ants, which are among the most invasive insects worldwide, establish following the entry of single nests or queens (Holway et al. 2002). This is the case in *W. auropunctata* whose population in Israel originates from one queen and one male genotype, that reproduce clonally (Vonshak et al. 2009). A similar pattern has been seen in Hawaï and in central Africa where single-clone introductions gave rise to the vast majority of local infestations (Mikheyev et al. 2009).

Its invasive success is highly associated with its particular reproductive system. Some populations have a classical haplodiploid reproductive system in which diploid females (i.e. queens and workers), are produced via sexual reproduction, whereas haploid males develop from unfertilised eggs through arrhenotokous parthenogenesis (Foucaud et al. 2007). In other populations, both queens and males are clonal (Fournier et al. 2005) but differ in their mode of reproduction. Diploid queens reproduce through automictic thelytokous parthenogenesis, a system showing strongly reduced recombination rates (Foucaud et al. 2010b) by which new reproductive females (gynes) are genetically identical to their mother.

Interestingly, this reproductive system is strongly associated with the type of habitat. Sexual populations are usually not numerically dominant (i.e. with low density of workers, brood, queens and nests), and establish mostly in natural environments with little or no human disturbance (e.g. primary or secondary forests), whereas clonal populations are usually numerically dominant (i.e. with high density of workers, brood, queens and nests) and colonise human-modified habitats (Foucaud et al. 2009). Therefore, low genetic diversity does not seem to be a barrier to establishment.

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response:
As shown with interception data from countries such as US (Bertelsmeier et al. 2018), New Zealand (Harris et al 2005), *W. auropunctata* is intercepted at ports of entry but not with a high frequency. However, the recent detection of established populations in Israel and Spain suggests a non-negligible rate of propagule pressure.

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
If we consider the invaded area in the risk assessment area, the maximum scores are given as a recent establishment of an active population in southern Spain (Espadaler et al. 2018) confirms the certainty of *W. auropunctata* establishing populations in the risk assessment area.

However, if we consider the uninvaded area, the scores decrease to likely with a medium confidence level as the predicted area covered by suitable conditions is restricted.
In the Mediterranean biogeographical region, establishment under current conditions is likely at least in urban areas (Spain, France, Italy, Greece, Croatia). Also, both the southern Atlantic (Southern France, Northeast of Spain and entire coast of Portugal) in the Mediterranean region and parts of Ireland and west of France are considered to be potentially susceptible (Bertelsmeier et al. 2015a Annex I; Beckmann et al. 2019 Annex IV). However, all these predicted suitable areas are restricted and cover a very limited area in the risk assessment area. When considering irrigation, suitable areas cover a larger part of the risk assessment area including biogeographic region (Federman et al. 2013, see annex II).

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.
With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Under foreseeable climate change, the overall area suitable for *W. auropunctata* will not significantly decrease in the future (according to Bertelsmeier et al. 2015a and Beckmann et al. 2019, Annexes I and IV respectively). *Wasmannia auropunctata* may shift from a southern distribution to a Northwest distribution. Whereas suitable areas are expected to decrease in Portugal, Spain, Italy, Greece, they will increase in Ireland and UK in the model developed by Bertelsmeier et al. (2015a). Beckmann et al. (2019) found an increase in suitable areas under foreseeable climate change in the Mediterranean, Atlantic and Black Sea Biogeographical regions (Annex IV).

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic "spread" via release or escape ("jump-dispersal"), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	**minor**	**CONFIDENCE**	**high**

Response:
Polygynous colonies spread by "budding", i.e. queens disperse only short distances over land and take workers with her to start a new colony. Such a strategy does not allow a rapid spread but does allow increased nest densities by increasing survival rates of queens and colonies. Such a pattern is currently observed in the newly infested area in southern Spain (Espadaler et al. 2018).

New colonies can also be founded by winged queens, capable of flying long distances. However, although winged queens have been captured in the invaded range (see above), independent queens have not been observed founding new colonies (Causton et al. 2005).

The question is scored "minor" because it is very likely to spread more slowly by natural means than by human assistance.

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.

- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	**major**	**CONFIDENCE**	**high**

Response:
Human assisted pathways of spread include the agricultural and horticultural trade of plants, plant materials, and soil/substrate as well as other movements of commodities and these are frequent and large.

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name:
a) Transport-Contaminant (Contaminant nursery material)
b) Transport-Stowaway (Container/bulk, including road transport, sea freight, airfreight, train, etc.)
c) Transportation of habitat material (soil, vegetation, wood, …)
d) Food contaminant (including of live food)
e) Unaided (Natural dispersal)

See question 1.1 for details.

a) Transport-Contaminant (Contaminant nursery material)

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Within Europe, movements of potted plants are unrestricted. Soil/substrate in potted plants is a favourite medium for nesting (see introduction and entry sections above). Thus, newly founded nests or parts of fully developed nests could easily be moved. Other horticultural material such as mulch, hay and other plant material can also harbour ant nests.

Polygynous nests include many queens and may contain thousands of workers. Ant nests might get onto the pathway in large numbers as contaminants of horticultural materials including soil.

The peculiar, almost unique, reproductive caste system of these eusocial insects can facilitate the development of viable colonies. For example, in the case of the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient to originate a colony (Hee et al. 2000; Luque et al. 2013).

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
A newly founded nest or parts of fully developed nests are able to survive transport and storage. The introduction of a population of *W. auropunctata* in Israel, which is believed to originate from south America, illustrates its capacity to travel over long distance (Vonshak et al. 2009).
Colonies of the ant *Temnothorax rugatulus* can survive for several months without food resources (Rueppell and Kirkman 2005).
Likelihood of survival is high, nevertheless will decrease with increasing travel duration. Multiplication of a colony (production of sexuals and reproduction) during spread within the EU cannot be ruled out, but is rather unlikely.

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Horticultural plants and products and soils/substrates are not systematically treated before translocation within the EU (directive 2000/29/CE) (see management annex for treatments before introduction into Europe).

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Fully developed nests could be quite visible even though workers are small (<2mm). In contrast, newly-founded nests with few queen(s) and workers can easily travel undetected in soil or other horticultural products.

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Potted plants and plant materials are often planted or stored in, or close to, highly suitable habitats, such as gardens, parks, road sides, etc. It is expected that the distribution of these media will facilitate occurrences in urban, suburban and agricultural habitats.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**medium**

Response:
We consider this pathway as the most likely pathway of spread of *W. auropunctata* within Europe. A similar conclusion has been made for New Zealand (Harris et al 2005).

The rate of spread will depend on the internal volume of trade within Europe.

For information, accidental transportation by humans has resulted in rates of spread of 10.50 km/yr in the case of *S. invicta* into uninvaded areas of the USA (Ross and Trager 1990).

b) Transport-Stowaway (Container/bulk, including road transport, sea freight, airfreight, train, etc.)

Qu. 4.3b. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:
Virtually any article of commerce can host hitchhiking ants within nests of all sizes and ages, including newly-founded and fully developed nests. A free volume of 10ml should be sufficient for an incipient colony composed by a queen and a dozen of workers. There are very many transported items (e.g. vehicles (incl. used car parts), machinery, building material, agricultural equipment packaging materials, bark, used electric equipment, non-agricultural soil, sand, gravel) that are suitable to carry nests and are grouped here together.

Qu. 4.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
There are very limited data on ant nests translocated within the EU. Polygynous nests include many queens and may contain thousands of workers. Ant nests might get onto transported items in large numbers as stowaways.

For the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient for a colony to grow quickly (Hee et al. 2000; Luque et al. 2013).

The likelihood of reinvasion after eradication is identical to the likelihood of spread in the first place.

Qu. 4.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
The likelihood of colony survival is high, but will decrease with increasing travel duration. Post introduction distances and hence transport periods are likely to be relatively short. Multiplication of a colony during spread within the EU cannot be ruled out, but is rather unlikely.

Qu. 4.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Most potential commodities that can carry ants or nests are not managed to limit ant spread.

Qu. 4.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Fully developed nests are quite visible. In contrast, newly-founded nests with few queen(s) and workers can easily travel undetected in most potential transported items.

Qu. 4.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**medium**

Response:
Several of the potential commodities and items in which nests can hide can be transported to suitable outdoor habitats since the ant particularly likes disturbed soils, which are found everywhere, specifically in urban, semi-urban and agricultural habitats.

Qu. 4.9b. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**rapidly**	**CONFIDENCE**	**medium**

Response:
Given the high numbers and types of commodities and items that can be associated with *W. auropunctata*, this species has the potential to spread rapidly in the RA area through this pathway.

The rate of spread will depend on the internal volume of trade within Europe.

The native range of *W. auropunctata* appears to have spread southwards over the past 60 years, as its southernmost distribution limit has recently been reported to be 34˚51’S in central Argentina, two degrees of latitude higher than those recorded previously (Chifflet et al. 2018).

c) Transportation of habitat material (soil, vegetation, wood, …)

Qu. 4.3c. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response: There should be no intentional spread of this species along this pathway.

Qu. 4.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Within Europe, movements of habitat (e.g. soil and vegetation) are unrestricted. Soil/substrate is a favourite medium for nesting as the species can nest in the soil. Thus, newly founded nests or parts of fully developed nests could easily be moved. Other habitat material such vegetation, can also harbour ant nests.

Polygynous nests include many queens and may contain thousands of workers. Ant nests might get onto the pathway in large numbers as contaminants of habitat material.

The peculiar reproductive caste system of these eusocial insects can facilitate the development of viable colonies. For example, in the case of the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient to originate a colony (Hee et al. 2000; Luque et al. 2013).

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 4.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
A newly founded nest or parts of fully developed nests are able to survive transport and storage. The introduction of a population of *W. auropunctata* in Israel, which is believed to originate from south America, illustrates its capacity to travel over long distance (Vonshak et al. 2009).
Colonies of the ant *Temnothorax rugatulus* can survive for several months without food resources (Rueppell and Kirkman 2005).
Likelihood of survival is high, nevertheless will decrease with increasing travel duration even if this pathway might concern only transfer over short distances (within member states). Multiplication of a colony (production of sexuals and reproduction) during spread within the EU cannot be ruled out, but is rather unlikely.

Qu. 4.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
There is no specific regulation along this pathway as invasive ants are not listed as pests.

Qu. 4.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Both fully developed nests and newly-founded nests with few queen(s) and workers can easily travel undetected in soil or vegetation as this pathway can involve large volumes of habitat material.

Qu. 4.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Habitat materials are often deposited in, or close to, highly suitable habitats, such as gardens, parks, road sides, etc. It is expected that the distribution of these media will facilitate occurrences in urban, suburban and agricultural habitats.

Qu. 4.9c. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**medium**

Response:
We consider this pathway as a likely pathway of spread of *W. auropunctata* within Europe. However, the transfer of habitat materials may occur mainly over short distances within a country which will limit the rate of spread within the risk assessment area.

For information, accidental transportation by humans has resulted in rates of spread of 10.50 km/yr in the case of *S. invicta* into uninvaded areas of the USA (Ross and Trager 1990).

d) Food contaminant (including of live food)

Qu. 4.3d. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response: There is unlikely to be any intentional spread along this pathway.

Qu. 4.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Within Europe, movements of food sources are unrestricted. Newly founded nests or newly-mated queens, although independent colony foundation has never been observed in the introduced range of the species, could easily be moved.

The peculiar reproductive caste system of these eusocial insects can facilitate the development of viable colonies. For example, in the case of the Argentine ant, *Linepithema humile*, it was shown that as few as 10 workers and a queen are sufficient to originate a colony (Hee et al. 2000; Luque et al. 2013).

The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

Qu. 4.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**Very likely**	**CONFIDENCE**	**high**

Response:
A newly founded nest or new-mated queens are able to survive transport. The introduction of a population of *W. auropunctata* in Israel, which is believed to originate from south America, illustrates its capacity to travel over long distance (Vonshak et al. 2009). Moreover, alive individuals have been intercepted at ports and airports in US on Yam tubers, *Zea mays* and *Zingiber officinale*. Ant interceptions on food represent 34% of the total records in US.

Colonies of the ant *Temnothorax rugatulus* can survive for several months without food resources (Rueppell and Kirkman 2005).

The likelihood of survival is high, nevertheless this will decrease with increasing travel duration. Multiplication of a colony (production of sexuals and reproduction) during spread within the EU cannot be ruled out, but is rather unlikely.

Qu. 4.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response:
Fruits and vegetable are often washed before shipment but there are no known existing management practices under current regulations during transport and storage. Fruits and vegetables can be infested after treatment either before departure or during transport. There is little information available on management during transport or its efficacy.

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
A newly-founded colony of a queen(s) and workers or a solitary queen can easily arrive undetected.

Qu. 4.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Food sources are likely to be transported indoors in warehouses, which may adjoin a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion. This is supported by the high propensity of *W. auropunctata* to invade urban areas even in its native range (Vonshak et al. 2010; Chifflet et al. 2018).

Qu. 4.9d. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**medium**

Response:
We consider this pathway as a likely pathway of spread of *W. auropunctata* within Europe. The rate of spread will depend on the internal volume of trade within Europe.

For information, accidental transportation by humans has resulted in rates of spread of 10.50 km/yr in the case of *S. invicta* into uninvaded areas of the USA (Ross and Trager 1990).

e) Unaided (Natural dispersal)

Qu. 4.3e. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	**unintentional**	**CONFIDENCE**	**high**

Response:

Qu. 4.4e. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:

Spread by budding includes a large number of workers and few queens that is sufficient to originate a viable population. This type a reproduction increases nests densities but limits the distance of spread to a few meters (Hölldobler and Wilson 1990).

The likelihood of reinvasion after eradication is identical to the likelihood of spread in the first place.

Qu. 4.5e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
Dispersion by budding increases queen survival compared to the low life expectancy of independent colony foundation.

Qu. 4.6e. How likely is the organism to survive existing management practices during spread?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:
There are no management practices currently in place.

Qu. 4.7e. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response:
Low ant densities (e.g. small newly-founded nests) often remain undetected for longer periods. However, spread will mainly occur from well-established nests, which would be more noticeable and spread should be detected earlier.
The fact that *W. auropunctata* has a painful sting, and is highly likely to be found in close association with urban areas, people should aid early detection of its presence, even if its initial establishment goes unnoticed (Espadaler et al. 2018).

Qu. 4.8e. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response:

Dispersion by budding is limited (less than 300m/year) (Holway et al. 2002) increasing the chances of individuals to find suitable habitats. This is particularly true in *W. auropunctata* which is a true generalist species that is able to invade both open and closed habitats.

Qu. 4.9e. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**very slowly**	**CONFIDENCE**	**high**

Response:
The rate of spread is relatively low in polygynous colonies that reproduce by budding (below 300m per year, Hölldobler & Wilson 1990). In Spain, the new population has spread across 5ha approximately in more than 5 years (Espadaler et al. 2018). Expansion rates of *W. auropunctata* vary from 73 m/year in Gabon (Walsh et al. 2004) and up to 500 m/year at Galápagos Archipelago (Lubin 1984).

For polygyne *S. invicta*, the invasion front moved 10.40 m/yr in central Texas via budding (Porter 1988).

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	**very difficult**	**CONFIDENCE**	**high**

Response:
It will probably be very difficult to physically contain the species. Its spread will be constrained by climate, habitat suitability and competition from other invasive species. If *W. auropunctata* become established in a European region, quarantine measures could be put in place to restrict the risk of medium to long-distance spread, e.g. through nursery stock, as in USA for *S. invicta.*

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**high**

Response:
Based on the low ecoclimatic suitability in Europe, we can estimate that it will spread unaided to all potentially infested biogeographical regions, but slower than in tropical and sub-tropical regions. However, recent studies confirmed the southern expansion of its native range, highlighting its capacity of adaption increasing potentially its suitable range in the risk assessment area.

Its spread will occur mainly through human transport but its distribution will be indirectly constrained by climate, habitat suitability and competition from other dominant ants (invasive and native).

The rate of spread will also depend on the internal volume of trade within Europe.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	**moderately rapidly**	**CONFIDENCE**	**high**

Response:
Climate change will not significantly increase the potential or speed of spread directly, even if it is expected to increase the distribution range to north-western Europe (Bertelsmeier et al. 2015a) (Annex I). Beckmann et al. (2019) found an increase in suitable areas under foreseeable climate change in the Mediterranean, Atlantic and Black Sea Biogeographical regions (Annex IV).
It may facilitate population growth with subsequently increasing potential for spread.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	**Major**	**CONFIDENCE**	**high**

Comment:
Wasmannia auropunctata is one of the most harmful invasive ant species worldwide. Indeed, the environmental impacts of *W. auropunctata* seem to be more pronounced than those of other invasive ants, except maybe the Red Imported Fire Ant *Solenopsis invicta* (Lowe et al. 2000; Holway et al. 2002). The severity of impact is most likely to relate to the population densities achieved.

Environmental impacts caused by the ant in the invaded ranged excluding the risk assessment area are multiple:

- <u>Impacts on fauna</u>:

The invasion of *W. auropunctata* is systematically followed by a reduction of biodiversity initially through a major decrease in ants and other invertebrates (Lubin 1984; Holway et al. 2002; Lach et al. 2010; Berman et al. 2013a,b). In addition to dominating many ant communities numerically, *W. auropunctata*, seriously impacts native ant communities with a systematic eradication of almost 100% of the native species (Orivel et al. 2009; Berman et al. 2013a,b). The effects of competition and predation alter the invertebrate community if the establishment of *W. auropunctata* at a site increased the total biomass of ant predators.

Foraging ants also prey on vertebrates and are a severe threat to vertebrates. The venomous sting of *W. auropunctata* may give it a greater ability to subdue vertebrate and large invertebrate prey (It has significantly reduced population sizes of endemic skink (Jourdan et al. 2001). It has also impacts on hatchlings of the Melanesian scrub fowl in northern Melanesia (Wetterer & Porter 2003). However, no studies that quantified impacts of *W. auropunctata* on vertebrate populations were found.

-Impact on plants:
The impact on wild plants has been less studied than that on animals or cultivated plants. *Wasmannia auropunctata* interferes with seed dispersal of myrmecochorous plants by reducing dispersal distances, and leaving seeds exposed on the soil surface (Ness and Bronstein 2004). These exposed seeds are not protected from fire or mammalian seed predators, and likely have less access to nutrients than do buried seeds or seeds deposited in ant nests.

As with other invasive ants, *W. auropunctata* is attracted to plants by their carbohydrate-rich resources or by honeydew-producing herbivores (Ness and Bronstein 2004). In the native range, they can provide protection to plants by molesting herbivores (De La Fuente and Marquis 1999). The presence of *W. auropunctata* benefits the plant: ant-visited plants grew significantly more in height than ant-excluded plants.

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?
Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	**Minor**	**CONFIDENCE**	**Medium**

Comment:
Because the species has only one newly established population in Europe, there is only one current study of its impact on biodiversity. Espadaler et al (2018) reported a decrease in native ants. However, a specific study monitoring the population dynamics and the associated impacts is needed to increase our confidence level.

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	**Major**	**CONFIDENCE**	**Low**

Comment:
It is likely that, if *W. auropunctata* spreads in the Mediterranean biogeographical region, the impact on native biodiversity, in particular on arthropods, and small vertebrates may be major to locally massive and similar to that it is observed in presently invaded areas elsewhere. These impacts would be at least similar to those of *Linepithema humile*, which is already spreading in the risk assessment area and threatens vertebrates (Alvarez-Blanco et al. 2017).

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:
N/A Because the species has only one newly established population in Europe, and there is no current study of its impact on biodiversity (except on the ant fauna) and related ecosystem services.

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	**major**	**CONFIDENCE**	**low**

Comment:

Wasmannia auropunctata can inhabit a wide range of habitats, open perturbated habitats to primary humid forest (Berman et al. 2013a). It is a threat to both invertebrates and vertebrates. In the risk assessment area, it will preferentially invade the Mediterranean biogeographic region which has the highest conservation value in the risk assessment area (Medail and Quezel 1999).

Therefore, many natural habitats of high conservation value, and their status, in suitable areas would be threatened by the ant. Some of them could be N2000 habitats.

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	**major**	**CONFIDENCE**	**medium**

Comment:
Provisioning-Nutrition: Foragers tend honeydew-producing homoptera, especially mealybugs, and including root feeding species. Souza et al. (2009) found that Homoptera were higher in areas of cacao plantations dominated by *W. auropunctata*. Homopteran tending often increases pest populations and can reduce crop seed set and yields. The presence of *W. auropunctata* was also associated with an increase in pest crop in Solomon islands subsistence gardens (Fasi et al. 2013). The presence and abundance of *W. auropunctata* therefore has the potential to inflict considerable crop loss in these rural subsistence gardens.

Regulating-Seed dispersal: *Wasmannia auropunctata* may interfere with seed dispersal activities of native ant species and therefore reduce the distribution of viable seeds (Ness and Bronstein 2004). They leave the seeds exposed on the soil surface, may ingest the elaiosome but fail to move the seed or they may move the seed shorter distances than the native ants they displace. These exposed seeds are not protected from fire or mammalian seed predators, and likely have less access to nutrients than do buried seeds or seeds deposited in ant nests.

Regulating-Pest and disease Control: *W. auropunctata* may interfere with beneficial insects that exert biocontrol activities in modified habitats. Although it has been introduced in Gabon to control agricultural pests, it is now no longer used because of its health impact. They were associated with higher pests abundance in cacao plantation and in subsistence gardens in Solomon islands (Souza et al. 2009; Fasi et al. 2013).

Cultural-Physical use of landscapes: *Wasmannia auropunctata* is a social nuisance in infested areas. *W. auropunctata* colonies are common around urban areas and are considered urban pests in many countries (see Harris et al 2005; Wetterer & Porter 2003). It could disrupt lifestyles, particularly outdoor activities that have a greater risk of contact with ants (e.g., picnics, gardening). Ant control would be necessary within a heavily infested area to allow such activities to continue.

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comment:
N/A. Because the species has only one newly established population in Europe, and there is no current impact on ecosystem services.

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	**Major**	**CONFIDENCE**	**medium**

Comment:
It is likely that, if *W. auropunctata* finds suitable habitats and climates for its development in the Mediterranean biogeographical region, the impact on ecosystem services may be major to potentially locally very strong and similar to that observed in presently invaded areas outside the EU. Although, its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability, its impacts in Israel are indicative for the risk assessment area.

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**major**	**CONFIDENCE**	**low**

Comment:
Wasmannia auropunctata is considered to be an economically important pest ant in some introduced areas however, data on the overall estimate of economic losses are unavailable.

Bueno & Fowler (1994) found that *W. auropunctata* was “the most consistently found native species” of ants found in Brazilian hospitals and “in inner portions of the hospitals, only the exotic species, and *W. auropunctata*, are consistently present.” *Wasmannia auropunctata* was the only native ant species not easily controlled with conventional insecticides. In southern Bahia, Delabie et al. (2006) found *W. auropunctata* in 12 of 100 houses inspected.

In many areas, *W. auropunctata* is a significant horticultural pest. It stings field labourers and they may be unwilling to pick fruit in infested areas (Smith 1965, cited in Harris et al. 2005). It enhances populations of honeydew producing homopterans, which are a pest in their own right and damage their host plant by sucking sap and encouraging the build-up of sooty mould (e.g., cocoa and citrus in Brazil (Souza et al. 2009), and citrus in Puerto Rico (Michaud and Browning 2006). The association between *W. auropunctata* and Homoptera may increase the occurrence of diseases, including viral and fungal infections, and in turn it increases the cost associated with agricultural pest management.

Fasi et al. (2016) showed that the presence of the little fire ant affects gardening activities by reducing time spent working effectively, influencing decisions about where to make gardening plots, discouraging children’s participation, and changing traditional gardening practices.

A notable eradication success in the early 2000s was the eradication of *W. auropunctata* from Marchena Island (Galapagos, 22 ha) (Causton et al. 2005). The eradication programme has cost approximately US$183,423, and a further US$136,000 was required for monitoring over the next four years. The total projected cost of removing *W. auropunctata* from one hectare of infested area was estimated in 2004 to be US$15,584 (Causton et al. 2005).

A recent study simulated the costs of decreasing or increasing management efforts to control *W. auropunctata* on the Hawaiian Islands (Lee et al. 2015). Since its introduction in the 1990’s it has spread to over 4000 locations on the island of Hawaii and has been found in isolated locations on Kauai, Maui, and Oahu Islands. This study demonstrated that increased management expenditures can suppress infestations; reduce spread between sectors; and decrease long-term management costs, damages, and stings.

Increased management effort has a significant impact on the number of Little Fire Ant sting incidents. Under current management, people on the island of Hawaii will suffer 2.3 billion sting incidents over 35 years. Their pets will endure 0.9 billion sting incidents over 35 years. With efforts to suppress Little Fire Ant populations, under least cost management during the next 35 years people and pets will suffer fewer sting incidents, down to 94 million for people and 9 million for pets. Management effort has a significant impact on costs and damages. In the next 35 years the cost of Little Fire Ant under current management will balloon to $6.1 billion. With efforts to suppress Little Fire Ant populations, under least cost management, net costs drop to $51 million, a substantial savings to the local economy.

An immediate expenditure of $8million in the next 2–3 years plus follow-up prevention, monitoring, and mitigation treatments will yield $1.210 billion in reduced control costs, $129 million in lowered economic damages, 315 million fewer human sting incidents, and 102 million less pet sting incidents over 10 years. Over 35 years, the benefits would include $5.496 billion in reduced control costs, $538million less economic damages, 2.161 billion fewer human sting incidents, and 762 million fewer pet sting incidents (Lee et al. 2015).

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found". Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:
N/A Because the species has only one newly established population in Europe, there is no current cost of damage but research costs have been incurred as a result of its arrival.

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comments:
It is likely that, if *W. auropunctata* spreads in the Mediterranean and Atlantic regions, the economic costs may be locally moderate to major, and similar to that observed in presently invaded areas elsewhere. Economic damages are sector-specific and vary with the size and extent of the infestation. Economic damages are based on estimated mean impacts from *W. auropunctata* and assumed to increase with level of infestation (Motoki et al 2013). However, its extent and strength which depend on the densities of ants, are very difficult to estimate considering the uncertainty related to habitat/climatic suitability. The economic damage in sector I at time t is:

$$D_{i,t} = c_i^{damage} \cdot \frac{{N_{i,t}^{final}}^2}{N_i^{max}}$$

Here C_i^{damage} is the average economic damage of an infested site in sector *I*, $N_{i,t}^{final}$ is the number of infested sites in sector I at the end of time t; N_i^{max} is the number of sites in sector I that are susceptible to *W. auropunctata*. Thus, when sector I becomes fully infested, $N_{i,t}^{final} = N_i^{max}$ and annual damage is $C_i^{damage} N_i^{max}$ (Motoki et al 2013).

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:
N/A Because the species has only one newly established population in Europe, there is no current cost associated with managing this ant.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comments:
It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the management costs may be locally moderate to major, and similar to that observed in presently invaded areas elsewhere. However, its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability.

A notable eradication success in the early 2000s was the eradication of *W. auropunctata* from Marchena Island (Galapagos, 22 ha) (Causton et al. 2005). The eradication programme has cost approximately US$183,423, and assuming that no more ants are found a further US$136,000 will be required for monitoring over the next four years. The total projected cost of removing *W. auropunctata* from one hectare of infested area was estimated in 2004 to be US$15,584 (Causton et al. 2005).

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**Major**	**CONFIDENCE**	**medium**

Comments:
Wasmannia auropunctata is a social nuisance in infested areas. Colonies are common around urban areas and are considered an urban pest in many countries (Harris et al 2005).
This ant has a painful sting that may cause injury to humans and domestic animals (Harris et al 2005, Lach et al 2010). The sting may produce an immediate, intense pain followed by red swelling.

Reports of widespread blindness in both humans and mammals caused by *W. auropunctata* stings deserve serious attention. The sting can cause irreversible corneal lesions leading to blindness (Rosselli and Wetterer 2017).

Bueno & Fowler (1994) found that *W. auropunctata* was “the most consistently found native species” of ants found in Brazilian hospitals and “in inner portions of the hospitals, only the exotic species, and *W. auropunctata*, are consistently present.” *Wasmannia auropunctata* was the only native ant species not easily controlled with conventional insecticides.

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comments:

It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the social impact, including health impact, may be moderate to potentially locally strong, and similar to that observed in presently invaded areas elsewhere.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	**minimal**	**CONFIDENCE**	**high**

Comments:
W. auropunctata is not known for being used as food or feed.

Ants have been observed carrying pathogens however up to date no transmission to Humans or food contaminations have been recorded (Alharbi et al. 2019). The score will be upgraded if evidence of transmission is produced.

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

Comments:
N/A - No other impacts were found.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	**major**	**CONFIDENCE**	**medium**

Comments:
There are no specialist natural enemies of *Wasmannia* spp. in Europe because there is no species of the genus *Wasmannia* in Europe. Thus, only generalist natural enemies of ants may affect the ant and these are highly unlikely to regulate (control) populations.

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	**major**	**CONFIDENCE**	**medium**

Comments:

It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the overall impacts, may be locally major, and similar to that observed in presently invaded areas elsewhere. There are strong assumptions that the species has already caused a decrease in local biodiversity in the risk assessment area (Spain), at least in the ant fauna and people are already complaining against its sting (Espadaler et al. 2018).

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	**major**	**CONFIDENCE**	**low**

Comments:

It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the overall impacts, may be locally major, and similar to that observed in presently invaded areas elsewhere. There are strong assumptions that the species has already caused a decrease in local biodiversity in the risk assessment area (Spain), at least in the ant fauna and people are already complaining against its sting (Espadaler et al. 2018). However, the extent of the impacts is very difficult to estimate considering the uncertainty related to habitat/climatic suitability.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Introduction*	**likely**	**high**	The species has been already recorded/intercepted in Europe and it is likely that this will happen again, specifically with contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freight. Climate change is not changing the risk of introduction or likelihood of entry based on the mentioned active pathways. Finally, queens may enter the risk assessment area through the ant market on the internet. This pathway should be considered in the future if the webmarket of ants is not sufficiently regulated.
Summarise Entry*	**likely**	**high**	The species has been already recorded/intercepted in Europe and it is likely that this will happen again, most likely via contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freights. Climate change will not change the risk of introduction or likelihood of entry based on the specified active pathways. Finally, queens may enter the risk assessment area through the ant market on the internet. This pathway should be considered in the future if the webmarket of ants is not sufficiently regulated.
Summarise Establishment*	**very likely**	**high**	Based on global species distribution models, *W.*

			auropunctata could become established in almost all countries around the Mediterranean Sea, with both the Atlantic Coast from Spain to Portugal and the Adriatic coast of Italy. Less than 2% of Europe is and will be suitable under climate change in the future to 2080. Predictions on the geographic extent of potential establishment indicate a slight increase in suitable areas.
Summarise Spread*	**moderately rapidly**	**high**	Based on the low ecoclimatic suitability in Europe, we can estimate that it will spread unaided to all potentially infested biogeographical regions, but slower than in tropical and sub-tropical regions. However, recent studies confirmed the southern expansion of its native range, highlighting its capacity of adaption increasing potentially its suitable range in the risk assessment area. Its spread will occur mainly through human transport but its distribution will be indirectly constrained by climate, climatic suitability and competition from other dominant ants (invasive and native). Climate change will not significantly increase the potential or speed of spread directly
Summarise Impact*	**major**	**low**	It is likely that, if *W. auropunctata* spread in the Mediterranean and Atlantic regions, the overall impacts, may be locally major, and similar to that observed in presently invaded areas elsewhere. However, its extent is very difficult to estimate considering

			the uncertainty related to habitat/climatic suitability. In other words, if only limited zones in the Mediterranean and Atlantic biogeographical regions will be favourable for the ant, impacts will be largely restricted to these zones.
Conclusion of the risk assessment (overall risk)	**high**	**medium**	*Wasmannia auropunctata* is one of the most damaging invasive ants on earth and one of the most successful at invading and colonizing new areas. There is no doubt that it can enter Europe through a variety of pathways, but its establishment and impact would be constrained by climate, habitat suitability and competition from other already established invasive ant species. It will have environmental, economic and social impact in some areas of Southern Europe, but the extent of its potential distribution remains unclear.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States (Based on Bertelsmeier et al. 2015a and Beckmann et al. 2019)

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria					
Belgium					
Bulgaria					
Croatia			Yes	Yes	
Cyprus					
Czech Republic					
Denmark					
Estonia					
Finland					
France			Yes	Yes	
Germany					
Greece			Yes	Yes	
Hungary					
Ireland			Yes	Yes	
Italy	Yes		Yes	Yes	
Latvia					
Lithuania					
Luxembourg					
Malta					
Netherlands	Yes				
Poland					
Portugal			Yes	Yes	
Romania					
Slovakia					
Slovenia					
Spain	Yes	Yes	Yes	Yes	Yes
Sweden					
United Kingdom	Yes		Yes	Yes	

Biogeographical regions of the risk assessment area (Based on Bertelsmeier et al. 2015a and Beckmann et al 2019)

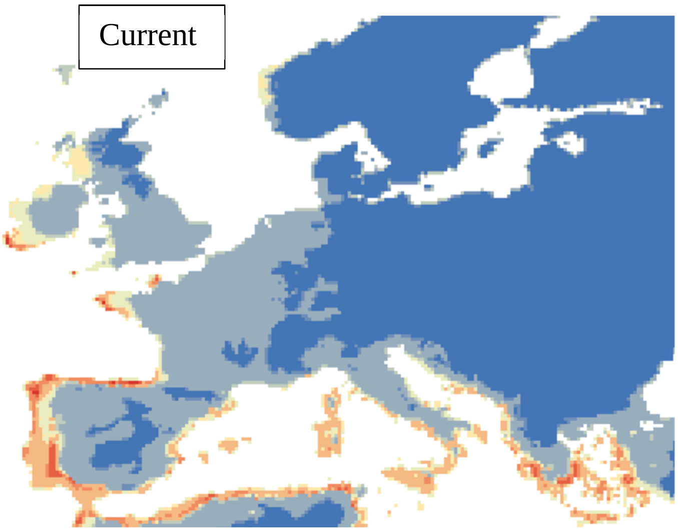
	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine					
Atlantic	Yes		Yes	Yes	
Black Sea				Yes	
Boreal					
Continental					
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian					
Steppic					

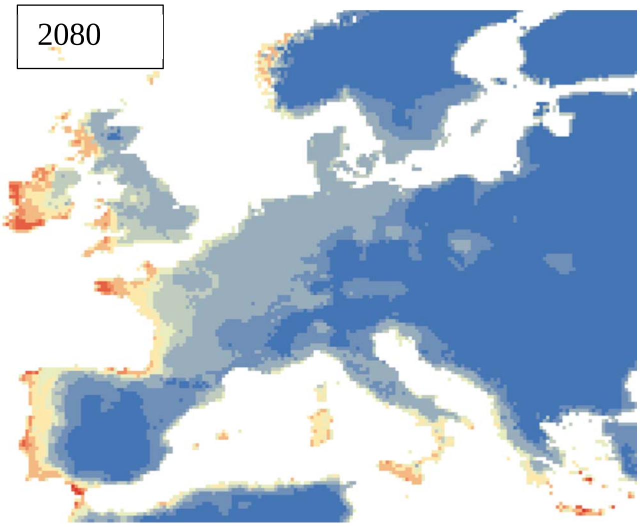
Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea					
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast					
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea					
Aegean-Levantine Sea					
Ionian Sea and the Central Mediterranean Sea					
Western Mediterranean Sea					

ANNEX I Species distribution models under current and future (2080) climatic conditions

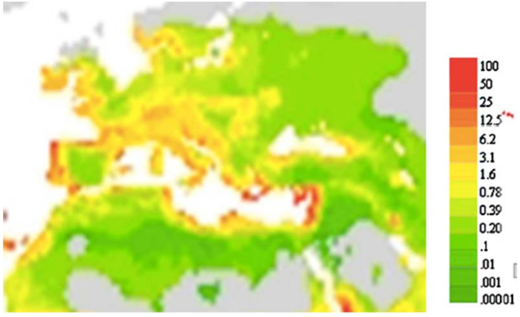
(source : Bertelsmeier et al 2015a). To consider a range of possible future climates, Bertelsmeier et al. (2015a) used downscaled climate data from three GCMs: the CCCMA-GCM2 model; the CSIRO MK2 model; and the HCCPR-HADCM3 model (GIEC 2007). Similarly, they used the two extreme SRES: the optimistic B2a; and pessimistic A2a scenario. They predicted an expansion of the potential range of *S. richteri* but the proportion of regions scored with a high suitability index (over 0.7) decreases. This method is based on the assumption that the species' niche remains unchanged when extrapolations are made in space (new potential distribution) and time (future climate scenarios). Occurrence points from both the invaded and native ranges were included to the full set of climatic conditions under which the species can persist because for invasive species in novel environments niche shifts can occur leading to differences with the native shift.





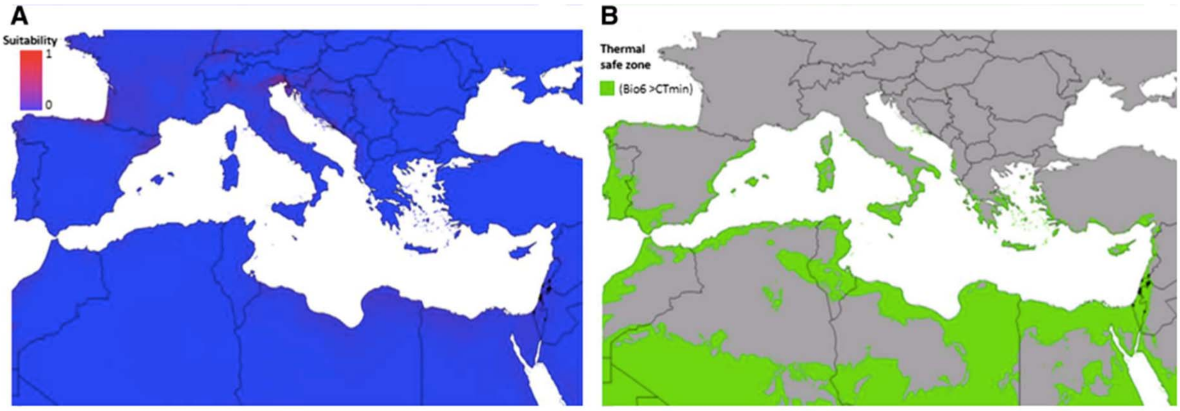
ANNEX II Species distribution models under current climatic conditions

(source : Federman et al. 2013). They used the Maxent model to predict potential invasion and establishment of *W. auropunctata*. Bioclimatic variables were obtained from the WorldClim dataset. These variables were derived from the monthly temperature and rainfall values, in order to generate biologically meaningful variables. The bioclimatic variables represent annual trends, seasonality, and extreme or limiting environmental factors. Yearly reference evapo-transpiration was obtained from the database of the Food and Agriculture Organization of the United Nations (FAO)



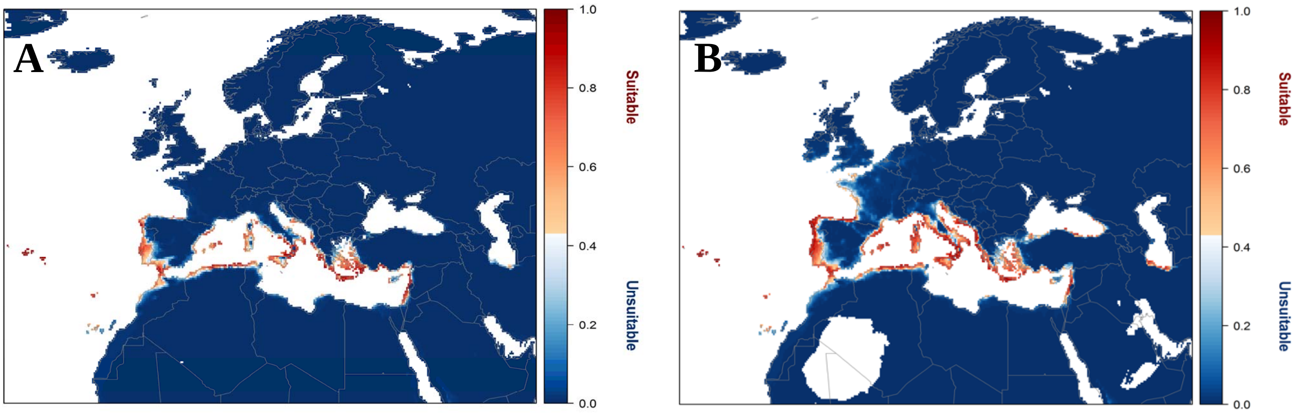
ANNEX III Climatic suitability and thermal safe zone maps (only Mediterranean basin)

a Maxent result with its habitat suitability index.
b Thermal safe zone map, in green regions with « minimum temperature of the coldest month » (Bio6) hotter than the lowest CTmin (4.2°C). *Wasmannia auropunctata* presences are depicted in black dots (source: Coulin et al. 2019). Coulin et al. (2019) analysed 19 bioclimatic variables related to temperature and precipitation at 30 arc-seconds resolution available from WorldClim. After variables selection, the remaining variables were analysed with *W. auropunctata* clade B native range presence data to fit a SDM using the Maxent procedure. To explore the link between the thermo-physiological constraints and the SDM, the lower CTmin measured in their study was evaluated by analysing the latitudinal change of the minimum temperature of the coldest month (Bio6) and its effect on the probability of presence.



ANNEX IV Projected (A) current suitability for *Wasmannia auropunctata* establishment in Europe and the Mediterranean region and (B) in 2070s under climate change scenario RCP4.6

(source: Beckmann et al 2019). To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip55m). Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats, we used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.



ANNEX V Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX VI Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[7]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread,

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[7] Not to be confused with "no impact".

	ecosystem effects			severe, long-term, irreversible health effects.

ANNEX VII Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX VIII Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)*
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties;

			Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[8]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*
		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control

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[8] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

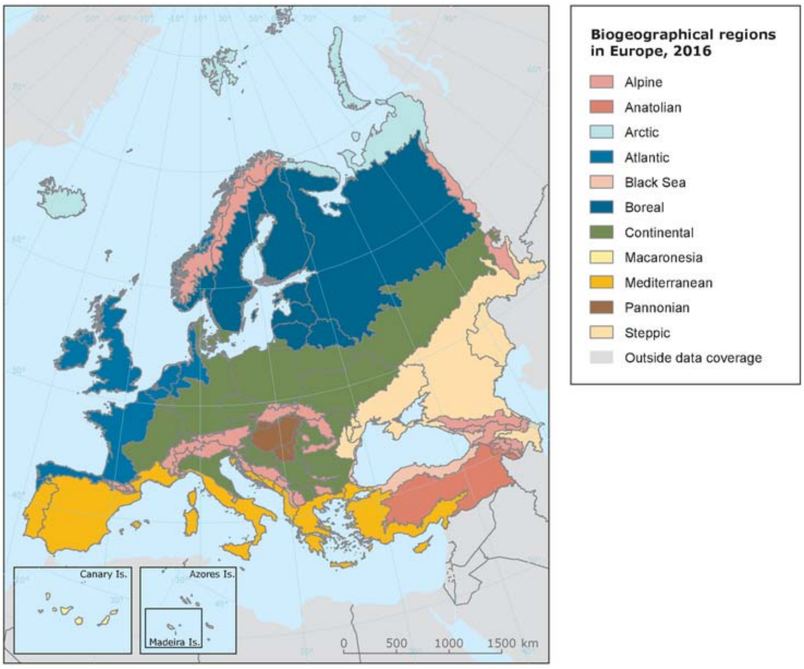
			Example: changes caused by non-native organisms to the abundance and/or distribution of pests
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

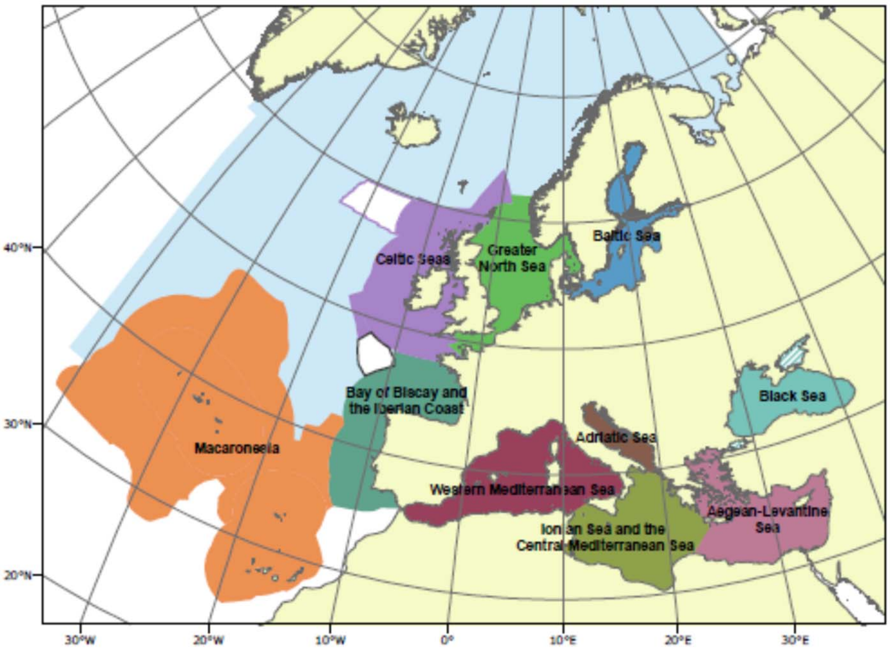
ANNEX IX EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeoregions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX X Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Wasmannia auropunctata*, Roger**
Species (common name)	**little Fire Ant**
Author(s)	**Olivier Blight, Marc Kenis**
Date Completed	**04/09/2019**
Reviewer	**Jørgen Eilenberg, Peter Robertson, Richard Shaw**

Summary [1]

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

To reduce the chances of establishment of exotic ants in Europe, it is necessary to prevent their accidental entry. Quarantine inspections and treatments methods used in other continents could be used in Europe. To do this, Europe needs to officially consider invasive ants as quarantine pests. The problem caused by invasive species should not exclusively be the concern countries of entry, but rather should be treated in collaboration to reduce risks of goods contamination. To increase efficiency in methods to achieve prevention, a careful inspection of goods at port-of-exit should be associated with active prevention at ports-of-entry. A careful inspection of the goods before shipment will decrease species dispersion and risks of invasion.

A successful eradication program is inseparable from an early detection of the infestation. Therefore, it is essential to develop contingency plans against this and other invasive ants at a European scale to be ready when ants are detected. European members should establish a list of ant specialists to whom the samples can be sent for rapid identification.

Given its very small size, *Wasmannia auropunctata* cannot be easily identified at inspection, surveyed and eradicated. Besides visual inspection, bait sticks and sniffer dogs can also be used for searching for *W. auropunctata* in inspected commodities. Only very small population in easily accessible areas can potentially be eradicated whereas larger populations should be the target of containment measures. Currently the most effective methods for eradicating, containing or control *W. auropunctata* are based on poisonous baits using various pesticides and attractants. Different types of baits are needed according to the nesting habitat, i.e. ground. vegetation or trees. It is important that insecticides are used in accordance with local legislations.

Cultural and sanitary methods and biological control also have the potential to contribute to integrated pest management approaches against *W. auropunctata* but more research is needed.

Detailed assessment			
	Description of measures[2]	Assessment of implementation cost and cost-effectiveness (per measure)[3]	Level of confidence[4]
Methods to achieve prevention [5]	**Inspection of imported goods and containers.** Goods, in particular soil, plants, wood, food and feed material from infested regions should be inspected at ports of entry. Because such systematic inspection is rarely possible, the selection of goods to inspect should consider their potential as a vector as well as their origin. Introduced ants are not drawn randomly from the biogeographic regions of the world (Miravete et al 2014; Bertelsmeier et al 2018). Most species intercepted in The Netherlands for example, had a Palearctic or Neotropical origin (Miravete et al 2014). Therefore, close attention should be paid to imports coming from these regions, especially the Neotropical region where *W. auropunctata* is present. However, invasive ants do not only arrive from the area of origin of the species but also via other localities (Bertelsmeier et al 2018). In particular, *W. auropunctata* may be present in shipments from	To reduce the chances of establishment of exotic ants in Europe, it is necessary to prevent their accidental entry. Inspection for ants should not be species specific but rather target invasive ants in general. At the global scale, the number of introduced ant species in temperate regions is considered to be three and half times higher than the number so far detected (Miravete et al 2014), which indicates the need to set up a common detection method at ports and airports at a European scale. In Europe, invasive ants are not officially considered as quarantine pests and, therefore, there is no legislation that specifically obliges quarantine services to identify, destroy and notify when ants are intercepted at inspections. Furthermore, inspection services in Europe are insufficiently equipped to cope with the vast and increasing amount of materials imported, resulting in only a small proportion of the imported material actually being inspected. An increased investment in manpower for inspection would be needed, combined with a more risk-based approach to better target high risk items if prevention of such invasions is to be achievable. To increase the efficiency of prevention efforts, a careful inspection of goods at ports-of-exit should be combined with an active prevention mechanism at ports-of-entry to prevent contamination. New Zealand is probably the most proactive jurisdiction preventing exotic species incursions; their biosecurity activities extending into four ports in three surrounding countries. This has proven to be efficient with a 98.5% reduction in contamination rates by ants of inbound goods within 12 months of active management (Nendick 2008). This system has led to reduced biosecurity contaminant and pest levels in New Zealand;	Medium

	European overseas territories such as Nouvelle Calédonie or French Polynesia, which are not regularly inspected. Considering its small size, *W. auropunctata* is not easily recognised by inspectors but all ant species, in particular queens and nests, should be destroyed immediately. Besides visual inspection, bait sticks and sniffer dogs can also be used for searching for *W. auropunctata* in inspected commodities (Vanderwoude 2014)	inspection actions have been significantly reduced, freeing staff for other vital work; significant cost reductions for importers and faster container clearance in New Zealand and less congestion in New Zealand ports as containers move off port faster. There is no information on the costs related to prevention methods for *W. auropunctata* but it is not believed that there would be any public concern over increased inspection activities for invasive ants and indeed their eradication in transit. There should also be no significant environmental or social harm at such a small scale of intervention.	
Methods to achieve eradication [6]	**Control at points of entry: Destruction of nests and ants found at inspection.** Hara et al. (2011) tested different methods to treat infested nursery plants including hot water drenches. When *W. auropunctata* infested potted plants were drenched with hot water (45.6°C) for 11 min, the number of live ants were reduced by 99.3 and 89.3% in rhapis and fishtail palm, respectively. Similar results were achieved with chemical compounds such as hydramethylnon, S-methoprene and metaflumizone. Irradiation is another quarantine treatment option to control ants on fresh horticultural products. Calcaterra et al. (2012) found that radiation doses >70 Gy stopped reproduction in *W. auropunctata* queens and should be sufficient as a phytosanitary treatment.	There is no information on the costs related to the destruction of nests and ants found at inspection but it should not exceed the cost of a chemical control of established nests.	Medium

	There are no specific guidelines on how to treat commodities infested by *W. auropunctata* at ports of entry, but guidelines developed for *Solenopsis invicta* (USDA 2010; USDA 2015) are largely valid for most invasive ants. Ant destruction can involve immersion or dip treatment, drench treatment, topical treatment and incorporation of granular insecticides into potting media.		
Methods to achieve eradication [6]	**Destruction of established colonies in the risk assessment area.** Eradication is possible against *W. auropunctata* but only when populations are still very small or on small islands. As for other invasive ants, eradications are conducted using toxic baits s of various nature. In the Galapagos, eradication programmes involved Hydramethylnon with soybean oil and hot dog and peanut butter were used as monitoring baits (Causton et al. 2005). In Maui Island, Hawaii, the ant was eradicated using three baits (Vanderwoude et al., 2010). Pyriproxyfen in crop areas and hydramethylnon in turf and ornamental areas and indoxacarb was applied to all vegetation >1.8 m tall (Vanderwoude et al., 2010). Three-dimensional treatment and repeated treatment are required	Few quantitative data on costs and cost-effectiveness of eradication campaigns are available. In Marchena Island, Galapagos, an eradication attempt on *W. auropunctata* covering 27 ha cost 0.213 million USD (Causton et al 2005). Examples include successful eradications of *W. auropunctata* on small islands of the Galapagos archipelago (Causton et al. 2005) or in Maui, Oahu and Kauai Islands, Hawaii (Vanderwoude et al., 2010, 2016). However, other eradication attempts in the same archipelagos and elsewhere failed (Causton et al. 2005; Wetterer and Porter 2003; Vanderwoude et al. 2016). The largest eradication attempt was conducted in Cairns, Australia, where an infestation of 28 ha has been under an eradication programme since 2006 and is still on-going (Kean et al. 2019). The total area under surveillance is now 24'350 km2 (Landcare Research 2018). The cost of this eradication programme of *W. auropunctata* started in 2006 is estimated to have cost AUS$ 9.9 million, most costs being borne by surveillance surveys (Landcare Research 2018). In an assessment for *W. auropunctata* in French Polynesia, Vanderwoude (2014) stated that eradication efforts should focus on eliminating small (<1 ha) infestations and if resources permit, infestations sized 1-5 hectares. In contrast, larger (>5 ha) infested sites	High

	for successful eradication of *W. auropunctata* (CABI 2019). Eradication and containment programmes cannot be successful without efficient surveillance and monitoring. These can be conducted using lure surveys, visual surveys, public reporting or detector dogs (see Vanderwoude 2014 for detailed methods)	should be the target of containment programmes. The same publication provides detailed advice on how eradication and containment should be conducted. In a recent meta-analysis, Hoffmann et al. (2016) established a cost-area relationship for ant eradication worldwide that could be relevant for *W. auropunctata* management. They compared two methods of bait application, by hand or aerial broadcast. The simulated cost to treat once 5 ha by hand was 2 500 US$ and around 8 000 US$ by aerial broadcast. Hoffmann et al. (2016) pointed out the necessity to conduct eradication on early detected ant populations as the success of an eradication is directly dependent on the area treated. Around 90% of the successful eradication campaigns were conducted over less than 10ha. There is no data on the public acceptance of these treatments but given the human health and nuisance impact of the ant stings many people would welcome their control though there will always be people who will resist the use of chemicals in the environment irrespective of the potential benefits. Regulations on the use of chemical pesticides in various environments will vary from Member State to Member State but in general use in urban environments and amenity land is less acceptable than arable fields	
Methods to achieve management [7]	**Chemical control.** Whether the aim is eradication, containment or control, insecticide-based methods are usually the only options. Chemical control will target not only the worker but also, and importantly, the queen, to kill nests. The most efficient option is the use of bait-formulated products. The most recent review and recommendations for products and	In Europe, similar control methods could be used, provided that the insecticides are registered in the country of application. Countries should have lists of chemical and biochemical insecticides authorised against invasive ants (as bait or contact) ready for using in case an invasion is detected. Chemical control is best when integrated into an IPM system that will limit its use to the minimum. Indiscriminate pesticide is not advocated. Non-target impacts must be weighed up carefully against the benefits of ant eradication.	High

	protocols for baits against *W. auropunctata* is found in Vanderwoude (2014) for French Polynesia. Treatment for the control of colonies nesting on the ground or in low vegetation (less than 1.5 metres) can be accomplished with commercial granular ant baits. However, *W. auropunctata* also nests in trees and higher vegetation, in which case non-commercial gel baits are more efficient. It is important that the use of insecticides in in accordance with local legislations. Hara et al. (2014) tested attractiveness of gel, granular, solid and paste of ant bait insecticides to *W. auropunctata*. Field attractiveness choice tests were conducted in an infested 37.2 m2 plot, and worker ant foraging and recruitment were recorded at 15-min intervals for 2 h. Granular and paste products that were as attractive as standard granular baits (Amdro Fire Ant Bait, Probait) included others formulated with hydramethylnon, abamectin, hydramethylnon and S methoprene, indoxacarb, fipronil, and metaflumizone. None of the gel or liquid ant bait products evaluated (active ingredients hydramethylnon, sodium tetraborate pentahydrate, thiamethoxam, fipronil or indoxacarb) were attractive to foraging workers. A list of eradication programmes carried out against other invasive ant species outdoors is provided in the	Data on the management costs of *Solenopsis invicta* eradication using insecticides in USA are available (Barr et al. 2005). Conventional bait insecticides cost approximately US$10 per 0.4 ha to broadcast apply, and with the cost of application, total treatment costs approximately US $17 per 0.4 ha (Barr et al. 2005) but treatment effects last only 3–12 months (Drees et al. 2013). Mound treatments with contact insecticides are much more expensive because *S. invicta* produces on average 168 mounds/ha (Porter et al. 1992). Such treatments are justifiable only in sensitive sites such as e.g. schools or sport fields (Drees et al. 2013) or after baits have largely reduced populations (Wang et al. 2013). A recent study simulated the costs of decreasing or increasing management efforts to control *W. auropunctata* on the Hawaiian Islands (Lee et al. 2015). This study demonstrated that increased management expenditures can suppress infestations; reduce spread between sectors; and decrease long-term management costs, damage, and stings. An immediate expenditure of $8 million in the next 2–3 years plus follow-up prevention, monitoring, and mitigation treatments will yield $1.210 billion in reduced control costs, $129 million in lowered economic damages, 315 million fewer human sting incidents, and 102 million less pet sting incidents over 10 years. Over 35 years, the benefits include $5.496 billion in reduced control costs, $538million less economic damages, 2.161 billion fewer human sting incidents, and 762 million fewer pet sting incidents (Lee et al. 2015).	

	GERDA database (Kean et al. 2017), which also lists techniques and products used for the eradication and references.		
	Cultural and sanitary methods. Cultural and sanitary practices can affect ant populations and be combined to baits in integrated approaches. In the Galapagos, fire and vegetation clearing were sometimes used to complement poisonous baits (Roque-Albelo and Causton 1999). More generally, improved land management, including improving land use efficiency and reducing the practice of monoculture, and a reduction in primary production are likely to reduce ant populations and the potential sources from new infestations (ISSG 2019). Niemiec et al. (2019) recently demonstrated that adding microinterventions to traditional outreach meeting motivated reputationally minded landowners to recruit and coordinate with other residents to control the invasive fire ant across property boundaries in Hawaii. This may have positive impacts on the species management.	There is no information on the cost-effectiveness of cultural and sanitary practices against *W. auropunctata*.	Low
	Biological control. The biological control of *W. auropunctata* has been considered but not yet tested. Wetterer and Porter	Wetterer and Porter (2003) described the process for a classical biological control programme against *W. auropunctata*. They estimate that a comprehensive program would likely require several hundred thousand dollars per year for 5-10 years. This estimate was based on	Low

	(2003) suggested considering classical biological control through the introduction of natural enemies from the native range, where populations are less abundant. Some natural enemies have been found in Central and South America, such as the eucharitid wasp *Orasema minutissima*, gamasid mites and unidentified fly larvae and microhymenopteran (Wetterer and Porter 2003). There is presently no pathogen-based biopesticide used against *W. auropunctata.*	experience with finding, screening, and releasing phorid flies as biocontrol agents for *Solenopsis* fire ants. They also state that a comprehensive biocontrol effort for *W. auropunctata* would probably require significant cooperative agreements between governments, conservation groups and scientific organizations concerned with the problem. Despite this, they consider that, though difficult and expensive, classical biocontrol is the only likely long-term solution to the ecological ravages of exotic populations of *W. auropunctata*	

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion. This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: *Axis axis* (Erxleben, 1777)

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Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: *Wojciech Solarz, Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland*
Peer review 2: *anonymous*

Date of completion: 6 February 2020

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

This risk assessment covers one species, axis deer *Axis axis* (Erxleben, 1777), also known as chital, cheetal, spotted deer or Indian spotted deer (Class: Mammalia, Order: Artiodactyla, Family: Cervidae, Subfamily: Cervinae, Genus: *Axis*).

Synonym(s): *Cervus axis* Erxleben, 1777.

According to Wilson and Reeder (2005) the genus *Axis* includes three species:

- *A. axis* in India (including Sikkim), Nepal, and Sri Lanka (plus a number of countries where the species is alien, see details in point A5 below);
- the Calamian deer *A. calamaniensis* (Heude, 1888), found in the Calamian Islands in the Philippines;
- the Indian hog deer *A. porcinus* (Zimmermann, 1780), known from Bangladesh, Burma, Cambodia, China, India, Laos, Nepal, Pakistan, Sri Lanka (perhaps introduced), and Vietnam, with introduced populations in Australia and South Africa.

Wilson and Mittermeier (2011) further include the Bawean deer *Axis kuhlii* (Temminck, 1836) in the genus, which other authors include in the genus *Hyelaphus*.

No subspecies of *A. axis* is recognised by Wilson and Reeder (2005) and Wilson and Mittermeier (2011).

There are no hybrids known to occur in the wild, however, as this cannot be completely excluded, under the precautionary principle this risk assessment should apply to all *A. axis* hybrids as well. Attempts to cross axis deer (*Axis axis*) with sika deer (*Cervus nippon*) by artificial insemination are reported (Asher et al 1999). One recorded case of hybridization arising from natural mating between sika deer and axis deer is also reported by Asher et al. (1999). In this case, the widest cross yet observed within the subfamily Cervinae, a hind exhibiting physical characteristics intermediate between the two species was born on a

Tennessee deer farm sometime in 1995. Electrophoresis analysis initially verified that hybridization had occurred, but fertility of the hybrid remained to be assessed. The potential for hybridization between axis deer and fallow deer (*Dama dama*) was explored by Willard et al. (2005), also using artificial insemination; in this case, reciprocal hybridization of the two species did not result in the establishment of hybrid pregnancies. Although anecdotal and undocumented accounts for the existence of such hybrids were reported (e.g. between sika deer and axis deer, see Bartos 2009), hybridization between these two species appears unlikely under natural conditions (Willard et al. 2005).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

The axis deer is a moderately large deer standing 88-97cm at the shoulders. Hinds are generally smaller than stags, that may weight up to 113 kg (and even over 136 kg in farms, see Centore 2016). Antlers are about 76 cm long and take roughly five months to fully develop (they are present only on stags). The species is characterised by a reddish brown coat covered with typical small white spots (retained at all ages and all seasons), arranged on the lower flanks in longitudinal rows. Under parts are white, as well as inner hind legs and under tail. A dorsal dark stripe is present from the nape to the tip of the tail (for further details see descriptions in Wilson and Mittermeier 2011, GISD 2015, Long 2003, Prater 1965). All the features mentioned above are useful to distinguish this species from other native deer in the risk assessment area. Otherwise only the prominent white throat is absolutely distinctive, because axis deer are in other respects not easy to distinguish (from superficial observation) from fallow deer or some spotted subspecies of sika. For example, in fallow deer younger bucks and does (especially of so-called ‘common’ or ‘menil’ coloration) do not have the distinctive palmated antlers which are typical of mature bucks, therefore they might be confused with axis deer in fleeting observation. Likewise, some colour variants of sika deer and pure Japanese sika deer in summer coat could be confused. Both common-coloured fallow deer, and sika deer, also have a darker dorsal stripe and dark line extending down the tail.
Albino animals are also occasionally reported (Dinesan et al 2006, Leo Prabu et al. 2013).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

A risk assessment for the axis deer exists for Poland (Okarma et al. 2018). The result shows that the risk for the country is considered “Medium” and the species is considered moderately invasive on the ground of its impact on the environment. In relation to the risk assessment area, this result may be considered valid particularly for the Continental biogeographic region.

The species was also assessed by Nentwig et al. (2018) according to whom this species is ranked 31 in the list of the “100 worst” alien species in Europe, arranged according to their impact (following the generic impact scoring system GISS, as calculated by Nentwig et al. 2010, which takes into account both environmental and economic impacts).

In Australia, a risk assessment for the species was made in Western Australia. The risk of establishing populations in the wild and the risk of becoming a pest have been assessed as “extreme” (Massam et al. 2010, Page et al. 2008). The map of Australia included in the risk assessment shows the partial suitability of the Mediterranean climate area for the species (Page et al. 2008). In Western Australia the species (either captive or released animals) was also considered as moderately dangerous” in relation to public safety, e.g. in relation to the potential for zoonoses, deer-vehicle collisions, injuries following aggressive behaviour (Massam et al. 2010, Page et al. 2008).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

The axis deer is a tropical or sub-tropical species, native to Asia, endemic of the Indian subcontinent, i.e. India (including Sikkim), Nepal, Bhutan, Bangladesh and Sri Lanka (Long 2003, Wilson and Reader 2005, Duckworth et al. 2015).

Axis deer is typical of the grassland-forest ecotone (Wilson and Mittermeier 2011). As summarised by Duckworth et al. (2015) the axis deer thrive in a wide range of habitats throughout its native range (see also Moe and Wegge 1994), but prefers moist and dry deciduous forest near water, interspersed with dry thorn scrublands or grasslands (Eisenberg and Seidensticker 1976). Mangrove forests (Sankar and Acharya 2004), mixed forests or plantations (with Teak *Tectona grandis* and Sal *Shorea robusta*) (Wilson and Mittermeier 2011) and agricultural crops such as coffee areas, are used too (Bali et al. 2007). This species lives mostly in flat areas and at lower elevations, usually below 1000 m, avoiding slopes, hills and mountain areas, but has also been found at high elevations (2,209 m) in India (Wilson and Mittermeier 2011, Duckworth et al. 2015, Schaller 1967, Deepan et al. 2018).

In general, this species avoids extreme habitats such as open semi-desert or desert, dense moist (evergreen) forests but introduced populations show some flexibility in this regard. For example, animals in the Andaman Islands are found in dense evergreen forests (Ali 2004, Sankar and Acharya 2004) and in Hawaii they are found in areas ranging from semi-deserts to rainforest (Moe and Wegge 1994), up to 2150 m (Waring 1996).

The native range is characterized by significant seasonal changes in temperature and, more importantly, extreme swings in precipitation (Anderson 1999), but axis deer have adapted very well to the European eco-climatic zones. For example, the typical habitat occupied in Croatia is represented by scrublands and woodlands of Euro-Mediterranean vegetation (Centore et al. 2018), while in Russia the species was successfully introduced to an area south of Moscow, characterized by deciduous and mixed forests with oak and undergrowth of spindle tree, buckthorn, dogwood, and other shrubs (Bobrov et al. 2008).

Four key factors were identified as delineating the axis deer's distribution: (1) the need for water; (2) the need for shade; (3) an avoidance of high, rugged terrain; and (4) a preference for grass as forage (Schaller 1967, Kushwaha 2018). Habitat use varies seasonally, reflecting food availability (see also Centore 2016). The axis deer easily habituates to human presence, and herds often congregate in open areas near habitation or forest camps to spend the night, possibly due to greater safety from predators and poachers (Duckworth et al. 2015). In fact, the limiting factor seems to be winter conditions, particularly strong frosts and thick snow cover (Okarma et al. 2018).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Outside the risk assessment area, the axis deer was successfully introduced in the following countries:

- in Europe (Moldova, Ukraine);
- Asia (Armenia, Azerbaijan, Andaman Islands, Pakistan, Ukraine);
- North America (USA: California, Florida, Texas and Hawaiian Islands, México);
- South America (Argentina, Brazil, Uruguay);
- Australia (for details, see Long 2003, Lever 1985, Wilson and Reader 2005, Duckworth et al. 2015, Wilson and Mittermeier 2011, Álvarez-Romero and Medellín 2005).

The species is also present in South Africa (https://www.invasives.org.za/legislation/item/709-axis-deer-axis-axis, accessed on 26/07/2019)

Axis deer were considered as introduced without success in New Zealand and New Guinea by Long (2003), while Forsyth and Duncan (2001) considered the introduction of this species as "successful" in New Zealand, because the species had a self-sustaining wild population before being eradicated by hunting. This shows that axis deer could have persisted in the climatic and environmental conditions in this country.

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

(6a): Alpine, Continental, Mediterranean
(6b): Mediterranean

The source of information on which the response is based can be found in Qu. A8.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

(7a):
Boreal, Continental, Mediterranean, Alpine, Atlantic, Black Sea, Pannonian, Steppic (see details in Annex VII).

(7b):

Boreal, Continental, Mediterranean, Alpine, Atlantic, Black Sea, Pannonian, Steppic (see details in Annex VII).

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.
A8a. Recorded: List Member States
A8b. Established: List Member States
Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom
The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

(8a): Czech Republic, France, Ireland, Slovenia, UK

Attempts to introduce axis deer were made as early as 1890 in France, but they did not succeed (Dorst and Giban 1954).

Axis deer was introduced to west-central Slovenia (from the Brijuni islands) in the late 1940s or in 1950, but this introduction failed (one stag, shot on 12 October 1950 is now in the Natural History Museum of Slovenia, see Duckworth et al. 2015). The species is considered as extinct in Slovenia (Mitchell-Jones et al. 1999).

According to Long (2003) axis deer were reported to be feral in Buckinghamshire, England, in 1944-45, but there is no evidence that they have been present outside a deer park and there is only one record of an escape by a single animal (hence it is dubious whether any population persisted in the wild). According to Fitter (1959) there were a number of reports of individual axis deer in England, but no evidence of breeding (one individual was shot in 1888 in West Sussex, and other animals were seen in 1944-45 in Combe at about the same time in other counties too).

Occasional records are available also for the following countries:

- Czech Republic: the species was considered as present in game reserves in the Czech Republic as early as 1850 (Mlíkovský and Stýblo 2006) but is now considered extinct (Nobanis 2019).
- Ireland (Fairley 1975).

(8b): Croatia (introduced in 1911)

The only wild populations in the EU are in Croatia, on the islands of Brijuni and Dugi Otok (Šprem and Zachos 2020, Linnell and Zachos 2011, Duckworth et al. 2015).

Axis deer are present on the Veliki Brijun island, the largest island in the Brijuni archipelago. Animals are free on the island, which has a surface of 560. Despite the fact that axis deer are described as capable swimmers (Nowak 1991), Axis deer has never been seen swimming from one island to another, unlike fallow deer (*Dama dama*), so it is considered that the population is restricted in these 560 ha (Public Institution National Park Brijuni and Hunting

Directorate of the Ministry of Agriculture, pers. comm. 2020). However, Šprem and Zachos (2020) mention that several cases had been reported of axis deer swimming from Brijuni Islands to the mainland (ca. 3 km), but establishment of new populations was unsuccessful. See also Qu. 4.1.

According to Centore (2016), the first introduction in Croatia dates back to 1911, when several individuals were introduced into Brijuni island from Germany (Šprem et al. 2008). The genetic origin of the introduced animals, however, is unknown (Kusak and Krapinec 2010). According to Long (2003) the population derived from animals which escaped from captivity in 1911 and have increased in numbers substantially. In 2008 the population in the Brijuni National Park reached about 100 individuals, was considered stable and rather numerous (Šprem et al. 2008). According to Šprem and Zachos (2020), some 150 individuals were present in the islands of Brijuni in 2017 (but the same authors also stated that up to 200 animals are removed each year for population control, which may create some confusion about the actual population size in the island). As of April 1, 2019, 76 animals were present according to the Hunting Directorate of the Ministry of Agriculture (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020).

In the area of Dugi Otok island, axis deer is currently present in two hunting grounds: a common open hunting ground number: XIII / 107 " DUGI OTOK – ISTOK" in which the number of axis deer is estimated at 10 individuals, and the state open hunting ground number: XIII / 4 " DUGI OTOK" in which the number is estimated at 12 individuals (as of April 1, 2019). Axis deer in these hunting grounds are not managed, but are removed from the wild in accordance to the relevant legislation (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020). According to Šprem and Zachos (2020) the existence of the axis deer population on the island of Dugi Otok originated in 2012 by 13 individuals escaped from a fenced area from the Brijuni Islands, and increased to about 60 individuals in 2018.

Additional introductions with animals from Brijuni were made in other parts of Croatia but did not succeed. For example, in 1953 the population introduced in the island of Cres declined gradually over the years, the last specimen being recorded in the early 1990s (Frković 2014). The species is considered well established in Brijuni, and according to Centore (2016) survived until the present day due to the favourable climate.

Long (2003) reports the introduction of two dozen axis deer released in Lithuania in 1954 (which reportedly adapted well and increased to 67 by 1961). However, this information seems not correct, and may well refer to sika deer (*Cervus nippon*) (see for example relevant information on Baleišis et al. 2003), in fact no information was found that axis deer has been ever introduced in Lithuania (Viktorija Maceikaite, pers. comm. 2019). In any case, no mention is made on the species in the review for Baltic countries made by Andersone-Lilley et al. (2010).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

(9a):
Austria, Belgium, Bulgaria, Croatia, Republic of Cyprus, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and the UK (see details in Annex VII).

(9b):
Austria, Belgium, Bulgaria, Croatia, Republic of Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and the UK (see details in Annex VII).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

The species is known to be invasive in several countries outside the risk assessment area (see review in Okarma et al. 2018). For example, in its alien range it is considered invasive in the Andamane Islands (Banerji 1955, Ali and Pelkey 2013, Mohanty et al 2016) and the US, i.e. in Hawaii (Anderson 1999, GISD 2015) and Texas (Long 2003), as well as in Argentina (Flueck 2009), and Russia (Bobrov et al. 2008).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Mediterranean: see answer to A12.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Croatia: At high population density axis deer was reported to cause significant damage in gardens, orchards and vineyards (Frković 2014). The species is mentioned as invasive in Croatia by CABI database (https://www.cabi.org/isc/datasheet/89941) but very little information was found on the impact on biodiversity. As reported by the Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture (pers. comm. 2020), it is difficult to say how much impact the axis deer itself has on the biodiversity of Brijuni National Park, but it is certain that mouflon and deer species significantly affect the biodiversity of Veliki Brijun islands where axis deer, mouflon and fallow deer are present. In the past, axis deer dominated over the other two species, but due to one harsh winter in the past many died and fallow deer has since prevailed. As the Public Institution National Park Brijuni has been reducing the number of deer specimens in recent years, currently mouflons are predominant. All three species together have a great impact on grasslands and forests of the island Veliki Brijun. Browsing and grazing of large herbivores that live on the island without natural predators affect lower layers of forests causing a problem for the natural reforestation and affect biodiversity of grassland allowing plants that the animals avoid (for example the Spanish oyster thistle *Scolymus hispanicus*) to overly spread.

According to the few data available from literature (see Šprem et al. 2008) *Axis axis* forages on *Fraxinus ornus, Quercus ilex* leaves and acorns, and sometimes browses the areas of *Myrtus communis,* new stems of blackberry *(Rubus spp.),* moss growing on rocks and cedar *(Cedrus spp.)* seeds. However, axis deer on the Brijuni Islands regularly consume supplementary feed such as hay and corn, regardless of the quality of the grassy areas (Šprem and Zachos 2020), therefore is likely that this prevents the species from having a greater (visible) impact on the island ecosystem. The evidence of higher impact may also be hidden by the fact that the populations in Croatia are all controlled through hunting (see Šprem and Zachos 2020). Always according to Šprem and Zachos (2020) axis deer impact on forest regeneration is less than other ungulates (i.e. European mouflon), but both terminal and lateral shoots are damaged.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and

an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

The axis deer is considered by some as amongst the most beautiful of all deer (Prater 1965) and this may explain the popularity in zoological gardens and parks around the world (Schaller 1967, Sankar and Acharya 2004). According to the European Association of Zoos and Aquaria, about 625 specimens were kept by zoos across 13 EU Member States[2] in October 2019 (EAZA, pers. comm. 2019). These numbers concern only zoos that are members of EAZA and can only provide an indication about the situation across the EU.

As summarised by GISD (2015) the meat of axis deer (venison) is highly regarded as it is extremely lean. It consistently ranks in the top ten of all venison in the world (Anderson 1999). As a result, there is an economic value for the meat.

The axis deer is also a prized hunting quarry, owing to its beauty, especially stags with antlers longer than 76 cm (although it was considered as an unattractive trophy animal in Croatia by Frković, 2014). Recreational deer hunting can thus provide both tangible and intangible social benefits (Jesser 2005). Many game ranches receive upwards of US$1000 for each trophy stag taken (Anderson 1999). In South Africa the costs for a trophy fee is €2,500 (http://www.fgsafaris.com/PriceList.htm, accessed on 26/07/2019). Poaching and black-market sales are common wherever the species occurs (Anderson 1999), and some documented evidence of skins and antlers seized from wildlife smugglers is available for India (TRAFFIC 2017).

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[2] Austria, Denmark, Czechia, Estonia, France, Germany, Hungary, Italy, Netherlands, Portugal, Spain, Sweden, United Kingdom.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided)”.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[3] and the provided key to pathways[4].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly

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[3] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[4] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

here, and there is no need to answer the questions 1.2-1.9

The following active pathways of introduction have been identified in the risk assessment area:

a) Hunting (Release in nature)
b) Farmed animals (including animals left under limited control) (Escape from confinement)
c) Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)

Another pathway known for the axis deer is "Landscape / flora / fauna "improvement" in the wild (release in nature)". However, this pathway is only known in regions other than Europe (no evidence was available for the risk assessment area). For example, in Australia, the establishment of wild deer populations began in the mid-1800s, when Acclimatisation Societies released deer for hunting or for aesthetic reasons (Moriarty 2004, Long 2003, Davis et al. 2016). The species was introduced as ornamental also in Argentina (Novillo and Ojeda 2008). As this pathway is considered not active in the risk assessment area, it is not considered further in this document.

The "natural spread" of individuals from neighbouring countries, e.g. Moldova, Russia, Ukraine (Long 2003, Duckworth et al. 2015) is another possibility. The likelihood of the species appearing in the natural environment of Poland as a result of expansion from Ukraine (near Dnipropetrovsk and in the Volga region, i.e. over 1000 km from the Polish border), however, was considered very low within the next 15 years (Okarma et al. 2018). Therefore, also this pathway, is not considered active in the risk assessment area, and not considered further in this document. Similarly, it is likely that some animals are kept as pets by private owners (for example in Croatia, according to the Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020). However, as no escapes are reported from this pathway, this is not considered active in the risk assessment area, and not considered further in this document.

a) Hunting (release in nature)

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**intentional** unintentional	**CONFIDENCE**	low medium **high**

This pathway refers to animals introduced into the risk assessment area to be hunted for food and/or to provide recreational hunting opportunities (including collection of hunting trophies). This is the typical pathway of introduction also in other regions, for example in Ukraine (Page et al. 2008), USA (GISD 2015), Argentina (Carpinetti and Merino 2000, Novillo and Ojeda 2008), Andamane Islands (Long 2003, Ali and Pelkey 2013, Banerji 1955), Australia (Massam et al. 2010, Moriarty 2004) and South Africa (https://www.invasives.org.za/legislation/item/709-axis-deer-axis-axis, accessed on 26/07/2019).

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through

this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Based on evidence relevant to past events, it is possible to expect further introductions and translocations of this species motivated by hunting purposes. Introduction is not expected to take place with large quantities (e.g. hundreds) of animals at one time. It would be expected, however, to be a number large enough to establish viable wild populations (considering that a number just above 7 animals is considered sufficient, see Qu. 2.3a.).

Several introductions occurred in Croatia, despite the unsuccessful result.
As summarised by Frković (2014) as part of an extensive programme of introductions to continental hunting grounds, axis deer were brought from the Brijuni islands into several sites in the Croatian Littoral in 1953. A number of factors, such as the inadequately organized capture and transport of the animals, the insufficient preparation of the introduction sites, the poor adaptation of the animals to new habitat conditions, the inability to roam, and the calf mortality in winter season, led to the failure of such introductions. The only site where the number of the introduced axis deer increased was in Punta Križa (island of Cres), where it was hunted as early as in 1955. However, due to the damage it inflicted to vineyards and households, the axis deer was hunted freely without any protection for several years (1965–1970). When the more attractive mouflon (*Ovis musimon*) and fallow deer (*Dama dama*) were introduced to the area in 1962 and 1966, the axis deer population of Punta Križa gradually declined over the years, so that the last specimen was recorded in the early 1990s. Therefore, the only wild populations still present in Croatia are those of the islands of Brijuni and Dugi Otok (Šprem and Zachos 2020)

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely	**CONFIDENCE**	low medium **high**

	very likely		

The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been successfully introduced in the past (e.g. in Croatia) and that secondary translocations occurred too. Hence, it is very likely that the animals survive during transport and storage along the pathway (provided appropriate animal welfare standards). The species is unlikely to reproduce or increase during such transport.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**N/A** very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

There are no management measures applicable during the introduction of animals.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**very unlikely** unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

The intentional introduction for hunting purposes cannot go undetected (although this is valid for authorised releases only, as any illegal introduction would likely go undetected).

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low medium **high**

The species was already introduced to the risk assessment area in the past along this pathway.

b) Farmed animals (including animals left under limited control) (Escape from confinement)

Qu. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**intentional** unintentional	**CONFIDENCE**	low medium **high**

This pathway refers to animals that have been introduced for farming into confinements, where they were kept with the primary purpose to provide food, resources and/or as working animals (it does not include animals held in zoos, deer parks and the likes, which are treated in the points below 1.2c to 1.7c). However, the number of axis deer farms present in the EU is unknown, and no information is available about the numbers of axis deer kept in such facilities.

The only documented evidence is a small population occurring in Croatia in a fenced area in the island of Rab (Centore 2016). In Germany, the species is kept in enclosures since 1707, although no occurrences are documented in the wild (Geiter et al. 2002, Nehring and Rabitsch 2015).

In Australia, the axis deer is the most popular farmed species among deer, and the most commonly released (Moriarty 2004). According to Massam et al. (2010), the species is used as livestock, e.g. for venison production, since the early 1800s in New South Wales (Moriarty 2004). In Texas the species occurs as a confined animal on ranches in 67 counties (Davis and Schmidly 1997, Long 2003).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been frequently kept in captive facilities. It is moderately likely that large numbers of animals are introduced for farming within one year.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport

and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

The likelihood of the animals to survive during transport and storage along the pathway is high, provided that appropriate animal welfare standards are ensured. Also, the likelihood of the axis deer to survive, reproduce, or increase in a fenced area is high, provided that the species requirements are duly considered and ensured (see for example Centore 2016, Centore et al. 2018).

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	N/A very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low medium high

The likelihood of the axis deer to survive existing management practices in a fenced area will vary depending on the type of deer management and extent of disturbance in the area. In principle it might be high, provided that the species requirements are duly considered and ensured (see for example Centore 2016). For example, as reported by Centore et al. (2018), the population in the fenced area in the island of Rab is actively managed through hunting. The hunting technique is stalking, distributed year round, depending on hunting season, and is characterised by an annual hunting bag of 6 animals (4 adults and 2 yearling) with a sex ratios of 0.86:1 in favour of stags. However, this is not deemed to affect the population, which in fact was specifically created and maintained for hunting purposes.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**very unlikely** unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

The intentional introduction for farming purposes cannot go undetected.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area

based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	CONFIDENCE	low medium **high**

The species was already introduced into the risk assessment area in the past along this pathway.

c) Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)

Qu. 1.2c. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**intentional** unintentional	CONFIDENCE	low medium **high**

Axis deer are known to be kept in zoos and wildlife parks for ornamental reasons. In fact this species was considered for centuries a favourite with zoological gardens and parks around the world (Schaller 1967, Sankar and Acharya 2004), and managed herds still occur in parks throughout the native and introduced range (Duckworth et al. 2015).

In Europe, the species is currently known to be present in captive facilities for ornamental reasons in many countries, like in the UK (Long 2003), Italy (Boitani et al. 2003), in Poland (Okarma et al. 2018), as well as in Denmark, Estonia, France, Croatia, Netherlands, Austria, Portugal, Sweden, Spain, Czech Republic, Cyprus and Germany (see https://www.zootierliste.de/?klasse=1&ordnung=121&familie=12110&art=1160403). According to the data from the European Association of Zoos and Aquaria (EAZA, pers. comm. 2019) taken from Species360 ZIMS the axis deer population in EAZA associated facilities is represented by 96 males, 236 females and 294 animals of unknown sex across 13 EU Member States[5] (information correct as of 03/10/2019).

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

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[5] Austria, Denmark, Czechia, Estonia, France, Germany, Hungary, Italy, Netherlands, Portugal, Spain, Sweden, United Kingdom

- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

It is moderately likely that large numbers of animals are introduced for keeping in zoos and deer parks within one year.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

The likelihood of the animals to survive during transport and storage along the pathway is high, as demonstrated by the fact that it has been frequently kept in captive facilities (hence provided that appropriate animal welfare standards are ensured). Also, the likelihood of the axis deer to survive, reproduce, or increase in a fenced area is high, provided that the species requirements are duly considered and ensured (see for example Centore 2016, Centore et al. 2018).

Qu. 1.5c. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**N/A** very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium high

There are no management measures applicable during the introduction of animals.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**very unlikely** unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

The intentional introduction into a zoological facility cannot go undetected.

Qu. 1.7c. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Axis deer is abundant in zoos and deer parks and the likelihood of further introductions or transport of animals between existing facilities (from outside the EU into the risk assessment area) is moderately likely.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.
Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low medium **high**

The species is already present in the risk assessment area through the described pathways, possibly leading to a risk of introduction in all biogeographical regions (but paucity of information on animals held in farms and parks does not allow to assess which regions exactly). It is to be noted, however, that apart from the one wild population in Croatia, itself restricted to an island, all current populations (by whatever route to date) are relative to animals held in confinement.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

There is no evidence that climate change will have any effect on the likelihood of introduction via hunting, farming or keeping animals in zoological facilities.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[6] and the provided key to pathways[7].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.
For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.
In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name:

The following active pathways of entry have been identified in the risk assessment area:

a) Hunting (Release in nature)
b) Farmed animals (including animals left under limited control) (Escape from confinement)
c) Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)

The “natural spread” of individuals from neighbouring countries, e.g. Moldova, Russia, Ukraine (Long 2003, Duckworth et al. 2015) is another possibility. However, as no detailed information is available on the exact location and relevant population size, or the population and expansion trends, this is not considered an active pathway for the time being and the relevant risk cannot be quantified.

a) Hunting (Release in nature)

Qu. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

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[6] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[7] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

RESPONSE	**intentional** unintentional	**CONFIDENCE**	low medium **high**

This pathway refers to animals released intentionally into the natural environment to be hunted for food and/or to provide recreational hunting opportunities (including collection of hunting trophies). The release for hunting purpose used to be the main pathway for the species in Europe, as documented in Croatia (Frković 2014; Centore et al. 2018), where several entries into the wild occurred, some of which with successful result (although ultimately only one population has been kept viable until present).

This has been a typical pathway of entry also in other regions, for example in Ukraine (Page et al. 2008), USA (GISD 2015), Argentina (Carpinetti and Merino 2000, Novillo and Ojeda 2008), Andamane Islands (Long 2003, Ali and Pelkey 2013, Banerji 1955), Australia (Massam et al. 2010, Moriarty 2004) and South Africa (https://www.invasives.org.za/legislation/item/709-axis-deer-axis-axis, accessed on 26/07/2019).

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low **medium** high

It seems that a very small number of hinds and a few stags is sufficient to found a new population. Despite some uncertainty regarding the outcome of the introduction of axis deer, the propagule size in deer introductions is considered a highly significant predictor of establishment success, as introduction involving four or fewer individuals failed, whereas involving seven or more individuals succeeded (Forsyth et al. 2004).

In Croatia the axis deer population is managed only in the fenced part of the state open hunting ground (number: VIII / 6 - "KALIFRONT") on the island of Rab, where a parental stock of 63 heads and an increase of 15 heads per year is defined by the game management plan (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020). This population originated from seven axis deer released in 1974 and resulting in a total of 78 animals during the 2015/2016 season, according to Centore et al. (2018). Axis deer in the island of Rab are all kept in a fenced area, but there are also animals reported out of the fence (Nikica Šprem pers. comm. 2020). However, any axis deer out of the enclosure needs to be removed from the wild in accordance to the relevant regulations (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020).

In other introductions occurred in countries outside Europe, the animals were subject to active management (i.e. hunting) therefore the data cannot be considered representative of any specific trend. However, several other introductions occurred in Croatia, although these were unsuccessful (see Qu 1.3a).

Outside the risk assessment area, in the Hawaiian Islands, deer populations flourished on Oahu, Molokai, and Lanai following releases. For example, as reported by Waring (1996) 8 axis deer (3 stags, 4 hinds, and one male fawn) were released in 1868 on Molokai Island where the population increased to 1,000 within 20 years and reached perhaps 7,500 before specific control measures were taken (see also Anderson, 1999). Similar trends were reported in other islands (Anderson 1999). In Queensland (Australia), one herd reported as still present by Bentley (1957) was established about 1866 by the introduction of a stag and two hinds. Similarly, in Rita Island (in Queensland) a population starting in the late 1970s from 20 individuals reached 2,000 or more in 2004 (Jesser 2005). In Ukraine, the number of axis deer increased from 25 individuals to 448 in 15 years (Anderson 1999), but no specific pathways are described. Also in Russia a population of axis deer grew rapidly, from 50 head in 1973 to 109 head in 1989 (Bobrov et al. 2008) but also in this case no specific pathways are described.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	low medium **high**

The intentional release of the species in the wild for hunting purposes cannot go undetected (although this is valid for authorised releases only, as any illegal introduction would likely go undetected).

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely **likely**	**CONFIDENCE**	**low** medium high

	very likely		

There is no documented evidence about which particular time of the year would be more appropriate for establishment. The diverse diet and habitats requirements along with the aseasonal reproduction patterns may open the window of opportunity for the entry of the species into the environment during most (if not all) months of the year. The likelihood of the animals to enter into the environment during the period most appropriate for establishment along this pathway therefore is high. Moreover, it is likely that hunters will release the animals in the most appropriate time and place, although there is no documented evidence that this has been systematically done (hence the low confidence).

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

Same as in 2.5a

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

The species has already entered the risk assessment area through this pathway, although there is no evidence that this is going to happen regularly.

b) Farmed animals (including animals left under limited control) (Escape from confinement)

Qu. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional **unintentional**	**CONFIDENCE**	low medium **high**

This pathway refers to the unintentional escape of animals from confinements where they were kept with the primary purpose to provide food, resources and/or as working animals. However, the number of deer farms present in the EU is unknown, and no information is available about the number of axis deer kept in such facilities.

Escapes from farms is a well known risk also in regions other than the EU, e.g. Ukraine (Page et al. 2008). In Australia, axis deer is the most popular farmed species among deer and the most commonly released (Moriarty 2004). According to Massam et al. (2010) escapes occurred since the early 1800s in New South Wales. Also, escapes from private captive facilities are reported in the US, particularly in Texas (Long 2003). In the USA, the origin a population introduced in the 1930s in Volusia County in Florida was caused by the escape from a private collection (Long 2003, Page et al. 2008).

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Although there are no specific data for axis deer (with the exception of some generic reference of animals escaped from fenced areas, see Šprem and Zachos 2020), escapes of other species of deer from farms are well known in Europe, as in the case of the sika deer (Bartos 2009). For example, in France an increasing number of small free-living sika deer populations have been reported to enter the wild (and establish) during the last decades, mostly as a result of escapes from deer parks (Baiwy et al. 2013) which share many analogies with deer farms. Also in Germany, according to Bartos (2009), frequent escapes of sika deer from an enclosure near Neuhaus, Möhnesee, occurred (here axis deer were present too, thus showing the inherent risk of entry associated to this pathway). Escapes of sika deer occurred also in Lithuania (Baleišis et al. 2003) and in Poland (Solarz et al. 2018).

Escapes of axis deer from farms are documented in other countries beyond Europe, e.g. in Australia (Jesser 2005). There, axis deer is known to be farmed since 1803, and already 6 years later the escape of 400 animals was recorded (Moriarty 2004).

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	low medium **high**

This is a medium sized deer heavily spotted in all seasons, and although mostly active around dawn and dusk (Wilson and Mittermeier 2011) it may be easily detected by hunters, naturalists, farmers, etc., hence it is unlikely to be introduced in the risk assessment area undetected. Nevertheless, the occurrence of other deer species throughout much of the risk assessment area may allow the entry of axis deer into the wild to go undetected by landowners and the general public not fully familiar with deer species differences.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

There is no documented evidence about which particular time of the year would be more appropriate for establishment, but is can be assumed that it is not during winter months (see for example limiting factors in Qu. 1.3a and 2.3a). The diverse diet and habitats requirements along with the aseasonal reproduction patterns may open the window of opportunity for the entry of escaped animals into the environment during most (if not all) months of the year.

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	**low** medium high

The likelihood of the animals to be able to transfer from the pathway to a suitable habitat in the environment through this pathway depends on the actual location of the deer farm. It is considered unlikely because of the lack of documented evidence on this regard, but on the basis of the experience with other deer species, it is not possible to exclude that this may happen.

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely **unlikely**	**CONFIDENCE**	**low** medium

	moderately likely likely very likely		high

The species has not yet entered the wild through this pathway, however, there is some risk for such events to happen as long as animals are kept in such facilities. For example in relation to the population on the island of Rab, as the species is known to be a good swimmer and move across islands by covering also distances of 10 km (see Qu. 4.1). However, the sound assessment of this point is affected by the lack of information about the distribution of deer farms in Europe where the species is held and their biosecurity.

c) Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)

Qu. 2.2c. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional **unintentional**	**CONFIDENCE**	low medium **high**

This pathway refers to the unintentional escape of animals from facilities such as zoological and deer parks where they are confined within enclosures, displayed to the public, and in which they may also breed. Nevertheless, a part from the number of zoos associated to EAZA, the total number of zoos and deer parks present in the EU is unknown, and no information is available about the number of axis deer kept in such facilities.

In Europe, there is no documented evidence on escapes of the species from captive facilities, except for some general references for the UK (Long 2003), but this possibility cannot be completely ruled out. In fact, escapes from private captive facilities is a well known risk in regions other than Europe, e.g. a release from a zoo in Armenia is reported (Long 2003), and escapes from captive facilities are reported too, e.g. for Ukraine and the US, particularly in Texas (Long 2003). Also the origin of the population introduced during early 1940s to Point Reyes Peninsula (Marin County, California) was the San Francisco Zoo (Long 2003, Page et al. 2008).

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	low **medium** high

No specific information has been found for axis deer, but it is considered unlikely that large numbers of animals escape from zoos or deer parks within one year.

Escapes of animals from deer farms and deer parks are however well known in Europe, as in the case of the sika deer (Bartos 2009). For example, in France an increasing number of small free-living sika deer populations have been reported during the last decades, mostly as a result of escapes from deer parks (Baiwy et al. 2013). According to Bartos (2009) frequent escapes of sika deer occurred in Germany, from an enclosure near Neuhaus, Möhnesee, where axis deer were present too, thus showing the inherent risk of entry associated to this pathway. Additionally, it is known that some populations of free-ranging fallow deer in Europe derive from escapes from deer parks. It is however unknown how many axis deer are kept in deer farms and parks in Europe. For example in Croatia, the Ministry of Agriculture (responsible for hunting) and the Ministry of Environment and Energy (responsible for nature protection) confirmed that they do not have any data or information on the existence of axis deer populations in held captivity (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020).

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	low medium **high**

This is a medium sized deer heavily spotted in all seasons, and although mostly active around dawn and dusk (Wilson and Mittermeier 2011) it may be easily detected by hunters, naturalists, farmers, etc. hence it is unlikely to enter the wild in the risk assessment area as an escape from a zoo or deer park undetected. Nevertheless, the occurrence of other deer species throughout much of the risk assessment area may allow the entry of axis deer to go undetected by landowners and the general public not fully familiar with deer species differences.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

There is no documented evidence about which particular time of the year would be more appropriate for establishment, but it can be assumed that it is not during winter months (see for example limiting factors in Qu. 1.3a and 2.3a). The diverse diet and habitats requirements along with the aseasonal reproduction patterns may open the window of opportunity for the entry of escaped animals into the environment during most (if not all) months of the year

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	**low** medium high

The likelihood of the animals to be able to transfer from the pathway to a suitable habitat in the environment through this pathway depends on the actual location of the zoological garden or deer park. It is considered unlikely because of the lack of documented evidence on this regard, but on the basis of the experience with other deer species, it is not possible to exclude that this may happen.

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

The species has not yet entered the wild through this pathway (as escapee from a zoo or deer park), but some risk for such events to happen exists as long as animals are kept in such facilities. However, the sound assessment of this point is affected by the lack of information about the distribution of zoos and deer parks in Europe where the species is held and their biosecurity.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant

biogeographical regions in current conditions.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low medium **high**

The most likely pathway of axis deer entry into the wild within the EU is the deliberate release for hunting (as it has happened in the Mediterranean biogeographical region in the past) and, less likely, the accidental/deliberate releases of individuals from deer farms and zoological gardens or deer parks.

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions. Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

There is no evidence that climate change will have any effect on the likelihood of entry via the active pathways.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low **medium** high

The only population established in the wild in the EU is in Croatia, on the islands of Brijuni, off Istria (Duckworth et al. 2015). However, environmental conditions similar to those present in the native and alien range of the species are present in other areas of the EU, particularly in the Mediterranean region, therefore it is likely that suitable sites exist elsewhere in all biogeographic regions in the risk assessment area (see Annex VII).

This may be partly confirmed by the fact that before being eradicated the axis deer was considered as successfully introduced also in New Zealand (Forsyth and Duncan 2001), a country partly sharing bio-climatic conditions similar to those found in Europe, as demonstrated by the many successful introductions of alien species of European origin.

Although native to tropical and subtropical areas of the Indian subcontinent, axis deer have adapted well to other ecoclimatic zones, including those present in the EU, such as the Mediterranean, and more continental climates in Russia and the Ukraine.

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	very isolated isolated moderately widespread **widespread** ubiquitous	**CONFIDENCE**	low **medium** high

Axis deer seem characterised by an extreme degree of flexibility and are well adapted to a wide variety of natural and semi-natural habitats and food, according to availability. Therefore, habitats or species (food sources) necessary for the survival, development and multiplication of axis deer are moderately widespread in the risk assessment area, particularly in the Mediterranean region.

Axis deer occupy a wide range of habitats throughout their native range, and are most commonly associated with a mixture of forest and more open grass-shrub, often avoiding rugged terrain, almost exclusively at lower elevations, below 1000 m a.s.l. (GISD 2015). Axis deer are typically associated with forest and grasslands interfaces but are highly adaptable to a wide range of habitats and changing conditions, including suburban settings (Duckworth et al. 2015). In particular, axis deer are found throughout dry and mixed deciduous forest habitat and secondary forest lands broken by glades, with a presence of understorey of grasses, forbs and tender shoots which supply adequate drinking water and shade. Axis deer consume an extremely wide variety of plants throughout their native and introduced range: about 160–190 of plant species (Duckworth et al. 2015, Sankar and Acharya 2004). Axis deer are predominantly generalist grazers that also browse leaves, flowers, fruits, and seeds, as well as bark when the preferred food items are scarce or during droughts (Anderson, 1999, Long 2003, Wilson and Mittermeier 2011 Duckworth et al. 2015, Schaller 1967), and possibly also during winter. Moreover, when natural forage is insufficient, axis deer forage in cultivated crops and cause economic damage (Anderson 1999). As summarised by Duckworth et al. (2015), axis deer is known to feed on mushrooms, crabs, rubbish and occasionally even human faeces in areas close to human habitation. Moreover, like in other deer species, antler and bone chewing is also common. The need to drink water once a day, more frequently in summer, in general restricts them to forest areas with assured presence of water, even if widely scattered.

However, the species is characterised by flexibility as shown by the significant seasonal changes in temperature and, more significantly, extreme swings in precipitation in their native range. These conditions force the species to deal regularly with long periods of drought and poor forage availability, as well as widespread flooding and lush seasonal growth during the rainy season (GISD 2015, Anderson, 1999). Outside its native range, in Hawaii, for example, axis deer is present from semi-deserts to rainforests (Moe and Wegge 1994).

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	**N/A** very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

No specific organism is required to be associated to axis deer for critical stages in its life cycle.

In their native range, axis deer are known to be associated with other animals, particularly monkeys, which produce alarm sounds on the presence of predators like leopard (*Panthera pardus*) or tiger (*Panthera tigris*) (Dinesan et al. 2006). However, this facilitative/mutualistic relationship is opportunistic and not obligate, and there is no evidence that this is required for critical stages in the life cycle of the species.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

There is potential for competition with the native red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), as well as other ungulates in the risk assessment area, but as noted for other introduced deer species such competition is unlikely to prevent establishment. However, no specific studies on axis deer exist in the EU on this regard. Studies carried out in regions outside the EU, e.g. in USA (Texas), showed aggressive and dominant behaviour in axis deer toward white-tailed deer (*Odocoileus virginianus*), demonstrating that species coexistence is unlikely, at least at the spatial scale of the study and depending on factors such as population density of the two species and habitat quality (Faas and Weckerly 2010).

Axis deer seem unable to tolerate the presence of feral pigs (Lever 1994), however explicit research on this possible relation is not available.

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

The enemies of axis deer in its native range vary from tiger to leopard, wild dog, jackal and python (Dinesan et al. 2006). In particular, jackals may kill fawns (Moe and Wegge 1994). As summarised by Sankar and Acharya (2004) in its native range in India the main cause of death is predation, mostly from tiger (*Panthera tigris*) and leopard (*Panthera pardus*). Outside the native range, predation was thought to limit the spread of axis deer, like in Australia, as a consequence of high density of dingo populations in some areas (Moriarty 2004). Wild boar (*Sus scrofa*) may also predate on axis deer fawns or juveniles, as reported in Argentina (Gürtler et al. 2017).

Other mortality factors in its native range are diseases (e.g. foot and mouth disease, rinderpest). The potential impact of an exotic epidemic like foot and mouth is demonstrated by the 1924 outbreak in California (Clements 2007). Also in Azerbaijan an introduced population was reduced by foot and mouth disease (Long 2003).

The risk assessment area is certainly characterised by the presence of potential predators, parasites or pathogens of axis deer, however there are several species of native and alien deer already occurring, and this does not seem to represent a limiting factor for their populations. Predation from large carnivores may be less effective in the risk assessment area, given the lack of tigers and leopards, and the potential impact of the large carnivores occurring in Europe is unknown. Therefore, natural enemies and diseases are unlikely to affect the likelihood of species establishment. Moreover, the role of predators in controlling ungulate populations remains uncertain, and is considered not effective, at least in some systems (Côté et al. 2004). The situation may be different in island ecosystems, where ungulates, as a consequence of their co-evolutionary history with large predators, may have very high reproductive rates, causing rapid population growth. For example in Hawaii, in the absence of predators, introduced populations of axis deer exhibit annual population growth rates of 20–30% (Hess 2008).

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Deer in Europe are usually subject to hunting and culling, which are regulated by law (see Apollonio et al. 2010). Poaching and overhunting has been a factor which led to the extinction of introduced populations of axis deer, e.g. in Croatia (Frković 2014). However, controlling axis deer may be problematic because it is a charismatic species, and there may be a conflict of interest between sectors obtaining recreational or economic gains from the exploitation of exotic wildlife and sectors promoting the conservation of biodiversity, as reported for Argentina (Gürtler et al. 2017).

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low medium **high**

Usually axis deer populations respond positively to higher levels of protection, water availability, forage quality, flat terrain and low predation, factors that are relatively widespread in the risk assessment area, although poaching and livestock grazing may be limiting factors (Duckworth et al. 2015). The availability of food and cover, which is usually provided to deer in game management reserves or in protected areas (where hunting may be forbidden, depending on the national legislation) may certainly favour the species establishment. Axis deer may benefit from water troughs established for cattle plus water sources on golf courses and homesites, as reported in Texas (Waring 1996). Also habitat restoration measures (i.e., prescribed burns and opening of fire breakers offering permanent pastures) may benefit axis deer (Gürtler et al. 2017). In addition, reducing competition (and perhaps predation) from wild boar due to its heavy hunting in the risk assessment area, may lead to an increase of axis deer abundance, as shown by a study assessing the result of a control program targeting both species in Argentina (Gürtler et al. 2017), which would increase the chance of successful establishment.

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	low **medium** high

There is no information for the risk assessment area, but overhunting has been a clear factor which led to the extinction of introduced populations (see for example Frković 2014). Nevertheless it is interesting to consider a long-term study of hunted axis deer in the introduced range in Argentina (for detail see Gürtler et al. 2017), which showed that contrary to park managers' expectations, the control program failed to reduce the axis deer population over a 10-year period despite increasing shooting effort and increasing deer harvest. Failure to reduce deer abundance may be explained by the combined effects of several putative processes: (1) population growth of axis deer over nearly two decades; (2) deer range expansion in the region leading to increasing immigration to the park; (3) sex- and stage-biased hunting mortality which kept per capita deer recruitment rates at sub-maximal levels, and (4) release from the pressure of wild boar (which was also a target of the control program) as a competitor (and perhaps as a predator).

Overall, the success of an eradication programme may depend on several factors, including the population size and the availability of resources. For example, in Russia the axis deer population of the Prioksko-Terrasny Nature Reserve (5,000 hectares) was reduced from 109 heads in 1989 to 5 in 2006 due to a control program (Bobrov et al. 2008).

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely	**CONFIDENCE**	low medium

	moderately likely likely very likely		**high**

Key biological characteristic which may facilitate the establishment of axis deer in the risk assessment area are the behavioural variability, opportunism and the species' extreme adaptability to changing circumstances (Anderson, 1999). As summarised by Duckworth et al. (2015) and references therein, the axis deer is a prolific breeder, which is documented by several empirical studies of the speed of increase by newly introduced subpopulations or in those where a factor restraining subpopulations was removed. For example, the population explosion in the Andaman Islands is considered a consequence of a series of factors (beside the presence of good vegetation) such as fast maturity, high annual pregnancy rate, low fawn mortality (Sivakumar 2003). In Bhadra, India, following the departure from the park of human settlements and consequent removal of anthropogenic pressures on axis deer and habitats, axis deer populations bounced back by nearly seven times in less than four years (Duckworth et al. 2015).

In the wild, axis deer are characterised by an aseasonal reproduction pattern (Centore 2016, Graf and Nichols 1966). The reproductive cycle of individual stags is not synchronised with that of other stags in the herd, hence they are found in rutting conditions throughout the year, do not retain harems and mate with hinds in more herds as they become receptive (GISD 2015). Hinds also experience non-synchronised oestrous cycles, with each cycle lasting about 3 weeks, and typically produce one fawn per pregnancy after a 210-238 days gestation period (Davis and Schmidly 1997).

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

The diverse diet requirements and the ecological flexibility which characterise the axis deer, along with the aseasonal reproduction patterns may facilitate the establishment of the species. Several other features of the species biology may explain the invasion success of the axis deer within the many introductions which occurred worldwide. For example, it is known to be a gregarious species, found in herds ranging from a few animals to 100 or more. In its native range, population densities fall within three to 50 animals per km^2 in India, up to around 200 axis deer per km^2 in Nepal (Duckworth et al. 2015). In Hawaii a herd as large as 300 was reported (Hess 2008). Natural lifespan of the species is 9-13 years, although zoo animals may reach 18-22 years (Davis and Schmidly 1997, Page et al. 2008).

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	very unlikely unlikely **moderately likely**	**CONFIDENCE**	low medium **high**

	likely very likely		

Apparently, a very small number of hinds and a few stags seems sufficient to fund a new population (which may show the negligible impact of genetic diversity), although no data on the impact of low genetic diversity in the founder population are available. See also **Qu. 2.3a.**

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur? Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

It is likely that high number of individuals are still kept and bred in captivity in the risk assessment area, which leads to a certain risk of some being intentionally or accidentally released in the wild, building up casual occurrences. The overall likelihood of casual population to occur seems low, but no sufficient data are available to support any statement on this regard.

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided. Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low **medium** high

Providing that sufficient founder individuals are encountered (see point 2.3.a), the axis deer is likely to establish self-sustaining populations in almost all EU Member States (with the exception of Estonia and Finland, see Annex VII) because appropriate climatic conditions, habitats and food are present and local natural enemies and diseases are unlikely to affect establishment.

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of

establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low **medium** high

Providing that sufficient founder individuals are encountered (see point 2.3.a), the axis deer is likely to establish self-sustaining populations in all EU Member States (see Annex VII) because appropriate climatic conditions, habitats and food are likely to be widespread (even more than in current conditions) and local natural enemies and diseases are unlikely to affect establishment.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic "spread" via release or escape ("jump-dispersal"), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	**minimal** minor moderate major massive	**CONFIDENCE**	low **medium** high

The potential of axis deer to spread within the risk assessment area by natural means is likely to be minimal, but there are no data about the rate of spread of individuals in Europe (which may vary depending on the extent of deer management and disturbance, as well as habitat availability and connectivity, appropriate food resources, presence of other species acting as competitor/predators etc.). For example, Okarma et al. (2018) pointed out that current information based on the lack of success of previous introductions in Europe and on biological characteristics of the species (size, life history, fertility, behaviour) allow to consider the spread rate of the population rather small.

Studies on spacing behaviour and habitat use in other parts of their native and alien range, show that animals are mostly sedentary and with small home ranges, usually between 180-890 ha (Long 2003, Moe and Wegge 1994), depending on resource availability (Waring 1996). Herds travel slowly at some 0.5 km/hour (Schaller 1967), but occasionally axis deer may make long trips to reach feeding grounds and water sources, for example during the dry season, and daily movements of up to 8 km for water have been reported (Graf and Nichols

1966). In Russia, the species was introduced approx. 100 km south of Moscow, in the Serpukhovskoe Hunting Reserve, and dispersed in about 10 years spontaneously to the Prioksko-Terrasnyi Biosphere Reserve through the Oka valley, just a few kilometres from the release site (Bobrov et al. 2008). In Queensland, although much of the area appears climatically suited to the species, axis deer were mostly concentrated surrounding their original release point, although drought may lead to wider dispersals of the animals (Jesser 2005).

Isolation of the axis deer in a small island, may not prevent the species from spreading. Axis deer are capable swimmers (Nowak 1991), and have been observed to swim fairly long distances between islands, i.e. about 3 km in Croatia (Šprem and Zachos 2020) and about 10 km in the Andaman Islands (Ali 2004, Ali and Pelkey 2003). In Brazil, the species is supposed to have reached the country from Uruguay by crossing the Uruguay River at the border between the two countries (Sponchiado et al. 2011).

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	**minimal** minor moderate major massive	**CONFIDENCE**	**low** medium high

The main (potential) pathway of spread is the natural dispersal across borders.

Otherwise axis deer were reportedly translocated and released intentionally in the risk assessment area for hunting purposes, e.g. in Croatia (Frković 2014). Moreover, the potential for spread after escapes from deer farms and deer parks should not be underestimated. The relevant introduction and entry pathways are already discussed in the corresponding sections above.

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Natural dispersal across borders of invasive alien species that have been introduced through pathways 1 to 5.

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional **unintentional**	**CONFIDENCE**	low medium **high**

This pathway is unintentional, as it depends on the dispersal capacities of the species. It is facilitated by the habitat conditions which characterise the area (including, for instance, the forest management regime and the recreational hunting practices, the extent of suitable ecological corridors etc.).

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely **likely**	**CONFIDENCE**	**low** medium high

	very likely		

No specific information is available on this regard. However as discussed in the sections above (see for example point 1.3a), it seems that in general a very small number of hinds and a few stags are sufficient to found a new population.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

The likelihood of the animals to survive, reproduce, or increase during spread (there is no transport and storage as such along this pathway) will vary depending on the extent of deer management and disturbance in the area (for examples in relation to land use practices, hunting, and other pressures).

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

The likelihood of the animals to survive existing management practices during spread will vary depending on the extent of deer management and disturbance in the area (for examples in relation to the hunting regime for ungulates).

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	low medium **high**

This is a medium sized deer heavily spotted in all seasons, and although mostly active around dawn and dusk (Wilson and Mittermeier 2011) it may be easily detected by hunters, naturalists, and farmers; hence it is unlikely to be spreading in the risk assessment area undetected. Nevertheless, the occurrence of other deer species throughout much of the risk assessment area may allow the spread of axis deer to go undetected by landowners and the general public not fully familiar with deer species differences.

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	**low** medium high

No information available on this regard. Based on information from similar species, animals dispersing through natural spread are highly likely to find suitable habitats for survival throughout the risk assessment area, except in areas devoid of any woodland (see GB Non-Native Species Secretariat 2011). The species would not spread by natural means along unsuitable habitats.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	very slowly **slowly** moderately rapidly very rapidly	**CONFIDENCE**	**low** medium high

Overall, natural spread from localised population is likely to be slow, but there are no data about the rate of spread of individuals in Europe (which may vary depending on e.g. the extent of deer management and disturbance).

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy **with some difficulty** difficult very difficult	**CONFIDENCE**	low **medium** high

Effective containment measures to prevent the spread of axis deer through the pathway above are the same as those to control/eradicate the species (see for example discussion on **Qu. 3.8.)**, hence their applicability is context dependent, and depends on the size of the population and the invasion stage.

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	very slowly **slowly** moderately rapidly very rapidly	**CONFIDENCE**	**low** medium high

See Qu. 4.9a.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly **slowly** moderately rapidly very rapidly	**CONFIDENCE**	**low** medium high

No information has been found.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	low medium **high**

Axis deer may cause significant direct impacts on native vegetation, e.g. through browsing and bark stripping, and may have a number of indirect effects on fauna and ecosystem processes. In general their impact (as an invasive alien species) is similar to that of other native deer, however it could amplify the pressure caused by ungulates on the natural environment. The impact may be even more sever where also other alien ungulates occur.

As summarised by Page et al. (2008) axis deer can feed on many species of native plants, as documented in the Hawaiian Islands (Hess 2008). Negative impact on natural regeneration of the native forests is also reported (Novillo and Ojeda 2008). In the Andaman Islands, where axis deer feed on over 70 different plant species (Sivakumar 2003), a negative impact on seedling and sapling survival, as well as on forest regeneration and forest structure is documented (Ali 2004; Ali and Pelkey 2013). In northern Patagonia, Argentina, introduced deer (among which axis deer) cause significant modification of the forest understory and impair the regeneration of canopy tree species (Veblen et al. 1989, Veblen et al. 1992), which seemed to negatively affect also the endemic conifer *Austrocedrus chilensis* (Relva and Veblen 1998).

Significant impact to individual trees which may limit the forests renewal is known to occur during the rut (reproductive season) when stags rub and wipe the antlers against the bark, frequently causing secondary infections, which may lead to the death of the trees, for example in Hawaii (Anderson 1999). Additionally, in extreme drought conditions (and possibly in winter) axis deer may feed on the bark of trees (Anderson 1999).

Another threat to the habitat and native vegetation may be caused by the deer trampling behaviour, which may lead to the creation of trails and increasing erosion and runoff (Hess et al. 2015, Page et al. 2008), for example in the Hawaiian Islands (Anderson 1999, Hess 2008). As summarised by GISD (2015) this results in a loss of the stability that vegetation provides, with resulting destabilisation of stream banks, subsequent changes in stream flow and increasing erosion and sedimentation of streams, ponds and rivers. When deer populations become very large, their trailing behaviour creates dirt paths even through the thickest of vegetation. These trails can lead to significant erosion and, in wet forest areas, increase runoff by decreasing the moss layer from soil that would normally retain water (Centore 2016, Anderson 1999). Soil erosion induced by the species leading to consequent siltation of offshore coral reefs is reported in Hawaii (Lever 1994).

Additionally, by opening up of habitat or by selective browsing of understory vegetation, axis deer could help in the spread and establishment of alien, and probably invasive, plants (Mohanty et al. 2016). Anecdotal observations exist that high axis deer densities lead to exposing bare ground, e.g. by removing the vegetation, which in turn may increase light levels and disrupt moisture dynamics, hence facilitating the invasion of exotic weeds (Jesser 2005, Davis et al. 2016). An example is the parthenium (*Parthenium hysterophorus*), a native to the New World accidentally introduced into several countries, including Australia, where it is flourishing in areas where axis deer are not adequately controlled (Jesser 2005).

Axis deer may also have a potential for endozoochoric dispersal of native and exotic plants, as documented in the case of the exotic hog deer (*Axis porcinus*) in south-eastern Australia (Davis et al. 2010).

Competitive displacement of native deer is another (potential) impact, as reported in Argentina (Novillo and Ojeda 2008). Axis deer is a generalist species, and scarcity of forage in the dry (or cold) season may lead to niche overlap with other cervids (Bhattarai 2019). For example, axis deer outcompeted white-tailed deer (*Odocoileus virginianus*) in experimental exclosures over an eight-year follow-up in Texas (Anonymous 2016). This study was within enclosures, where by definition competition may be enhanced because there is no opportunity to avoid competition through niche differentiation or use of species-specific refugia, therefore the results are only indicative (but may reflect situations in closed environment, e.g. small islands). Another research conducted in Texas showed aggressive and dominant behaviour in axis deer toward white-tailed deer, which subsequently modified the habitat selection and feeding patterns (Faas and Weckerly 2010). Axis deer may have a competitive advantage over white-tailed deer for being less specialized in food requirements, while the role played by the different susceptibilities to parasitic disease (Richardson and Demarais 1992). Another study carried out in an enclosure (although about the size of a small island or a small protected area) demonstrates that coexistence of these two species is unlikely, at least at the spatial scale of the studies and in any case depending on factors such as population density of each species and habitat quality (Faas and Weckerly 2010). Ferretti and Lovari (2014) stressed the difficulty to use an experimental approach in field conditions, but pointed out that evidence on overlap in the use of resources, opposing trends in population size, and behavioural interactions support the hypothesis of competition between alien ungulates and native ones. This however needs to be evaluated on a case by case.

Indirect effects on native biodiversity by altering ecosystem processes may be more subtle and affect also animals other than ungulates. For example, a study showed that in the Andaman archipelago axis deer depressed the abundance of forest floor and semi arboreal lizards approximately five-fold, by reducing vegetative cover in the understory (Mohanty et al. 2016).

Detrimental effects of axis deer are reported from outside the risk assessment area in relation to the conservation status of threatened species at the global level. This is mostly as a consequence of the habitat degradation, as documented by the IUCN Red List, in this case with examples limited to the situation in the Hawaii (BirdLife International 2016a, 2016b, 2016c, 2016d, 2018a, 2018b, 2018c, Bruegmann and Caraway 2003, Heddle 2004). For instance, this is deemed to affect four species that are considered Critically endangered (CR): the Pacific Lacefern (*Ctenitis squamigera*), the Olomao (*Myadestes lanaiensis*), the Maui Parrotbill (*Pseudonestor xanthophrys*) and the Ou (*Psittirostra psittacea*). Two additional species, the Maui Alauahio (*Paroreomyza montana*) and the Fabulous Green Sphinx Moth (*Tinostoma smaragditis*), are considered Endangered (EN). Because of its burrows trampled by axis deer and other ungulates, the Hawaiian Petrel (*Pterodroma sandwichensis*) is considered Vulnerable (VU). Axis deer also contributed to the destruction of the habitats of two extinct Hawaiian species (EX), the Black Mamo (*Drepanis funereal*) and the Bishop's Oo (*Moho bishop*).

Overabundant deer may apparently exert cascading effects on other animals by competing directly for resources with other herbivores and omnivores and by indirectly modifying the composition and physical structure of habitats of both invertebrates and vertebrates (Côté 2005). High deer abundance resulting from the introduction of alien deer species, may have strong indirect effect on forest birds through their impact on vegetation and associated insects. For example, as documented by Allombert et al. (2005) overabundance of white-tailed deer (*Odocoileus virginianus*) populations in North America, resulted in a decrease in songbird habitat quality through decreased food resources and nest site quality and may partly explain continental-scale decreases in songbird populations. An introduced herbivore may even lead to the indirect extirpation of an abundant large carnivore, as documented in a large island in Canada, where the near eradication of shrubs producing berries by introduced white-tailed deer (*O. virginianus*) was considered as the main cause of the extirpation of black bears (*Ursus americanus*) within approximately 50–70 years (Côté 2005). As a remark, the examples above which pertain to other deer species in countries other than the EU, and as such do not necessarily apply to axis deer in particular, especially if those deer are not at high densities. However, the information was deemed indicative for the purpose of this assessment, to show the diversity of impacts potentially emerging from the introduction of a new deer species in the EU.

Qu. 5.2. How important is the current known impact of the organism on biodiversity at

all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?
Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

No direct evidence of impact in the risk assessment area exists other than what is reported for the presence of the species in Croatia.

As reported by the Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture (pers. comm. 2020) it is difficult to say how much impact the axis deer itself has on the biodiversity of Brijuni National Park, but it is certain that mouflon and deer species significantly affect the biodiversity of Veliki Brijun islands (where axis deer, mouflon and fallow deer are present). In the past, axis deer dominated over the other two species, but due to one harsh winter in the past many died and fallow deer has since prevailed. As the Public Institution National Park Brijuni has been reducing the number of deer specimens in recent years, currently mouflons are predominant. All three species together have a great impact on grasslands and forests of the island Veliki Brijun. Browsing and grazing of large herbivores that live on the island without natural predators affect lower layers of forests causing a problem for the natural reforestation and affect biodiversity of grassland allowing plants that the animals avoid (for example the Spanish oyster thistle *Scolymus hispanicus*) to overly spread. According to the few available data from literature, in the island of Brijuni, axis deer are known to feed on grasses and ash (*Fraxinus ornus*) leaves and holm oak (*Quercus ilex*) leaves and acorns, and sometimes browse the leaves of myrtle (*Myrtus communis*), new stems of blackberry (*Rubus* spp.), mosses growing on rocks, and cedar (*Cedrus* spp.) seeds (Šprem et al. 2008). No information on the type and scale of impact is available. It must be noted that the island of Brijuni is characterised by a very intense human use, limiting the possibility of observing impacts on natural ecosystems of the axis deer.

A couple of studies were carried out in the hunting reserve in the Island of Rab (Krapinec 2002a, 2002b), but their results may be of limited applicability for the assessment of impacts in the wild, because the location was inside an actively managed forest in a fenced area.

Based on evidence from outside the risk assessment area it can be expected that overabundant deer may have a substantial impact on woodland vegetation (modifying patterns of relative abundance and vegetation dynamics), and play a significant role in woodland ecosystem function. In case axis deer would get established on islands, the impacts on the local ecosystems as well as on some bird species (e.g. petrels) could be severe. In the absence of control (either by predators or humans), deer populations can rise to very high densities. This may be further facilitated by human management of forests providing ideal habitats. Vegetation changes brought about by browsing and trampling axis deer are detrimental to other deer species as well as other vertebrate and invertebrate species (see note by Gill 2000). Cascading effects on other species may extend to insects, birds, and other vertebrates. Hence, axis deer may tip forest ecosystems toward alternative states by acting as “ecosystem engineers” or “keystone herbivores”, as generally noted for deer (Côté et al. 2004).

According to Okarma et al. (2018) in the worst case axis deer may locally cause hardy reversible changes in ecosystem functioning. According to Okarma et al. (2018) it can therefore be assumed that in the event of spreading in Poland, the impact could lead to serious decreases in the population size of some native protected species. In Poland, it can be expected that axis deer may exert a certain negative impact on native deer species, as also remarked by the Council of Europe (2002) for Croatia. The potential for competition with native deer may be particularly strong because such species have not shared a common evolutionary history. Additionally, some possible competition with European bison (*Bison bonasus*) may be expected in Poland, should axis deer become established and widespread in this country (Okarma et al. 2018).

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate **major** massive	**CONFIDENCE**	**low** medium high

In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly. Because there is no documented evidence the confidence is low.

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor **moderate** major	**CONFIDENCE**	**low** medium high

	massive		

The axis deer may represent a potential threat for a series of species and habitats protected by the Birds and Habitats directives, as well as a number of IUCN red-listed species, as shown in countries outside the risk assessment area. The effect of axis deer on protected species of plants and relevant habitats would reflect its browsing habits and diet, as well as the ability of the plants to withstand damage (including from trampling, etc.). Therefore, several plants may be susceptible to axis deer impact, not to consider the cascading effects that overabundant axis deer populations may apparently exert on other ungulates (see for the possible competition with *Bison bonasus*, which could occur if the species were to establish in Poland, Qu. 5.2) and other groups of animals as well, including birds on islands.

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly. In case of a future expansion of the species range, other native species may be affected. While there is no documented evidence of the species being able to cause the extinction of any native species, the level of risk is assessed as being “moderate” also in the future.

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.

- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

Axis deer may affect several ecosystem services, not only through the discussed impacts on biodiversity, but also due to the impacts documented on ornamental plants and agricultural crops through browsing and bark stripping, for example in the Hawaiian Islands (Hess 2008). The erosion caused by the trampling behaviour associated with the death of trees caused by the habit to wipe their antlers on the barks, may results in destabilisation of stream banks, changes in stream flow and increased erosion and sedimentation of waterways (Anderson 1999, GISD 2015). Additionally, it is known that the trailing behaviour has caused erosion and damage to a variety of culturally or archaeologically significant sites in Hawaii (Anderson 1999). The role of axis deer in the regulation of zoonosis, because of its pathogens and parasites, is another possible threat to both wildlife and livestock, and to humans (Okarma et al. 2018).

Here follows a list of potential impacts on ecosystem services (based on the CICES classification V5.1):

Provisioning (Biomass)

- Cultivated terrestrial plants
- Reared animals
- Wild plants (terrestrial and aquatic)
- Wild animals (terrestrial and aquatic)

Regulation & Maintenance (Regulation of physical, chemical, biological conditions)

- Baseline flows and extreme event regulation
- Lifecycle maintenance, habitat and gene pool protection
- Pest and disease control
- Soil quality regulation
- Water conditions

Cultural (Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting)

- Physical and experiential interactions with natural environment
- Intellectual and representative interactions with natural environment

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

No information has been found.

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

Comment:

In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly (at the moment there is no evidence of impact, but should the population grow and spread, the impact may become evident). As there is no documented evidence of the species being able to cause other types of impact, the level of risk can be expected to be “moderate” in the future. However, because of paucity of information, confidence of this assessment is low.

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	low medium **high**

As pointed out by Page et al. (2008), the species is capable of having an impact on sheep, cattle, cereal grain, grain legumes, and other fruit (pineapple) commodities. Crop damages by axis deer, particularly when other available forage is scarce, have been described in both the native and the introduced range (Anderson 1999, Hess et al. 2015, GISD 2015, Page et al. 2008). For example, in Hawaii severe and extensive damage to the pineapple industry on Lānai was reported (Lever 1994, Hess et al. 2015). In Maui, more specifically, deer were blamed to be responsible of an estimated $35,000 to $55,000 in crop losses to Maui Pineapple Co., and one farmer claimed about 40 deer caused US $20,000 in fence and corn crop damage in one night (Kubota 2001).

When overgrazing occurs, axis deer are known to compete with livestock and native wildlife (Long 2003). Being primarily grazers, axis deer compete for food mainly with domestic cattle and sheep (Lever 1994). In particular, direct competition for forage with cattle is reported in both California and in Texas (Anderson 1999). In California, in Point Reyes National Seashore, the cost to the park for staff, equipment, vehicles and supplies to monitor and manage non-native deer (both axis deer and fallow deer) was approximately $140,000, or 2.5% of the park annual budget (GISD 2015, National Park Service 2004). In Argentina, although regularly hunted, axis deer populations have increased in some provinces, interfering with livestock production (Flueck 2009).

Deer may transmit infectious diseases directly to livestock (as well as to other deer and to humans), especially if deer density is high (Côté et al. 2004). In particular, axis deer have been shown to carry and transmit bovine tuberculosis (*Mycobacterium bovis*) and several other diseases in both the native range, i.e. in India (Schaller 1967) and the introduced range. For example, in Hawaii, bovine tuberculosis was found in five percent of deer from Molokai, posing an ongoing threat to cattle trade throughout the islands (Hess et al. 2015). In California, in addition to carrying several livestock and wildlife diseases, a small percentage of axis deer also harboured Johne's disease (*Mycobacterium paratuberculosis*), a contagious bacterial disease of the small intestines of ruminants (Hess et al. 2015). In Russia, the species was considered responsible for the introduction of the deer louse fly (*Lipoptena cervi*) (Bobrov et al. 2008), although this parasite is considered native to the region. However, some studies suggest that the indigenous parasite fauna of small founder populations of introduced exotic ungulates, such as the axis deer in Hawaii, frequently does not persist in their free-ranging progeny and that subsequent parasite communities acquired from sympatric ungulates are of limited diversity and comprised primarily of species exhibiting a broad host range (McKenzie and Davidson 1989).

Besides carrying parasites and pathogens, axis deer are responsible for a number of deer-vehicle collisions, as regularly documented on Molokai, Hawaii (Anderson 1999, Page et al. 2008), however the economic impact is not quantified.

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found". Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal **minor** moderate major massive	**CONFIDENCE**	**low** medium high

No direct evidence of impact in the risk assessment area exists other than what is reported in Croatia. For example, in the island of Cres, the axis deer inflicted damage to vineyards and households (Frković 2014). No economic damage was recorded in the Island of Rab in a study aimed at the analysis of the feeding activities of axis deer and mouflon (*Ovis ammon*) in an actively managed (fenced) forest community of holm oak and manna ash (*Fraxino orni-Quercetum ilicis*) (Krapinec 2002a).

Okarma et al. (2018) considered that in case the species established in Poland, the impact of the species on crops would be "medium".

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly.

Overabundant deer are known to inflict major economic losses in forestry, agriculture, and transportation and contribute to the transmission of several animal and human diseases (Côté et al. 2004). See also comments in **Qu. 5.19** and **Qu. 5.10**.

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

No information has been found.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	minimal minor moderate **major** massive	**CONFIDENCE**	**low** medium high

In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the costs may be expected to increase accordingly. If the species spreads and is to be managed, some costs are bound to be incurred, even if there is no info on what these costs are currently. But it is not possible to estimate the monetary value, as it depends on deer management systems and policies involved, which vary considerably across the different countries of Europe depending on species present, legislation, cultural tradition and the status of deer as *res nullius* or *res communis*.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor **moderate**	**CONFIDENCE**	low **medium** high

	major massive		

Deer may transmit infectious diseases directly to humans (as well as to other deer and to livestock), especially if deer density is high (Côté et al. 2004). The axis deer, as other ungulates, can be a carrier of a number of diseases and parasites that may be harmful to humans. For example they carry common parasites that may directly affect humans, i.e. if droppings enter freshwater systems (GISD 2015). Parasitic zoonoses harbored by the species include: leptospirosis, cryptosporidiosis, and strains of *Escherichia coli* (Anderson 1999). A potential role of axis deer and their associated ticks (e.g. *Ixodes pacificus*) in the ecology of the Lyme disease spirochete, *Borrelia burgdorferi*, was evidenced through a study in California, USA (Lane and Burgdorfer 1986, Page et al. 2008). However the relationship between density of deer (and other large herbivores) in the environment and environmental tick burden is controversial, with different studies coming to different conclusions, hence the information above should be considered only indicative.

Overall, as pointed out for alien mammals in general (Capizzi et al. 2018), axis deer can act as vectors of both alien and native pathogens, and as host of either native or alien parasites (which in turn can be acting as vectors of either native or alien pathogens). In this way axis deer may either introduce new pathogens, alter the epidemiology of local pathogens, become reservoir hosts, and increase disease risk for humans, along with other species (e.g. by introducing changes in the vector-host-parasite relationship).

In addition to carrying diseases that can infect humans, axis deer may cause road collisions, e.g. as reported in the Hawaiian Islands (Hess 2008). On Maui roads, for example, at least 36 motor vehicle collisions with axis deer occurred during an 18-month period between 1999 and 2000, see http://archives.starbulletin.com/2001/08/28/news/story8.html).

An indirect human health issue that deer axis pose in Hawaii is the potential for stray bullets to hit people as poaching increases (Anderson 1999). In any case shooting for managing the species is considered potentially dangerous and has led to complaints as it may represent a safety risk for residents, e.g. mostly because is conducted at night, as reported in Australia (Mitchell 2015).

Axis deer is an animal that is unlikely to make an unprovoked attack but such attacks can cause serious injury (requiring hospitalisation) or fatality if animals are cornered or handled (Page et al. 2008).

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor **moderate**	**CONFIDENCE**	**low** medium high

	major massive		

In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

Deer, in general, may transmit infectious diseases directly to other species of deer (as well as to livestock, and to humans), especially if their density is high (Côté et al. 2004). The axis deer, as other ungulates, can be a carrier of a number of diseases and parasites that may be harmful to native species. For example, this species is involved in the transmission of bovine tuberculosis (Anderson 1999, Schaller 1967), which is a deadly disease for native ungulates, including the European bison (*Bison bonasus*) as pointed out by Okarma et al. (2018). Other diseases transmitted by axis deer in their native range are leptospirosis and cryptosporidiosis (Anderson 1999, Schaller 1967). The species may also act as a new host for native parasites, as in the case of the tick *Amblyomma dubitatum* found on axis deer in northern Argentina, and this interrelationship may have potential deleterious effects on the native fauna, due to acquisition and amplification of the native parasite by an introduced host (Debárbora et al. 2012).

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	**CONFIDENCE**	low medium high

No information has been found.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

As described in Qu.3.5 the risk assessment area is certainly characterised by the presence of potential predators, parasites or pathogens of axis deer. However, there are several species of native and alien deer already occurring here, and this does not seem to represent a limiting factor for the relevant populations (predation from the large carnivores may be less effective than in the native range, given the lack of tigers and leopards). In fact, the role of predators in controlling ungulate populations remains uncertain as pointed out by Côté et al. (2004), and is considered not effective, at least in some ecosystems.

The situation may be different in island ecosystems, where ungulates, as a consequence of their co-evolutionary history with large predators, may have very high reproductive rates, causing rapid population growth in the absence of predators. For example, in Hawaii, introduced axis deer exhibit annual population growth rates of 20–30% (Hess 2008).

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

The species is known to exert a multifaceted impact on both biodiversity and ecosystem services, by feeding on native vegetation and contributing to the loss of habitat structure and function (hence indirectly affecting other species, including birds, reptiles, invertebrates, etc.). Competition with other ungulates is documented. The species is known to contribute to the spread of diseases and pathogens affecting both livestock and humans. It can also damage crops and compete with livestock. It can be a threat in relation to possible deer/vehicle collisions. No documented exists to provide discuss in details the overall impact in the biogeographical regions.

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future

conditions.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

In foreseeable climate change conditions, the area suitable for the species in the risk assessment area may increase, and the impact may be expected to increase accordingly. For example, in case of a future expansion of the species range, other native species may be affected. No documented exists to provide discuss in details the overall impact in the biogeographical regions.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Introduction*	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The species is already present in the risk assessment area (in the wild and in confinements). Further introductions for hunting, farming or exhibitions are very likely.
Summarise Entry*	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Releases or escapes from captive facilities, have been documented in the past in the risk assessment area and are very likely to take place again.
Summarise Establishment*	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Axis deer, although native to tropical and subtropical areas of the Indian subcontinent, has the ability to establish in other ecoclimatic zones, including those present in the EU, such as the Mediterranean. In Croatia it is currently established. The species life-history, available habitat conditions and management practices in the EU offer the potential to support self-sustaining populations of axis deer also in other countries and biogeographical regions.
Summarise Spread*	very slowly **slowly** moderately rapidly very rapidly	low medium **high**	The species has a sedentary habit, but is also known to spread over some distance in specific circumstances (e.g. suitability of habitat, lack of predators), including across islands given the good swimming skills.
Summarise Impact*	minimal minor **moderate** major massive	low **medium** high	The species is known to exert a multifaceted impact on both biodiversity and ecosystem services, by feeding on native vegetation and contributing to the loss of habitat structure and function (hence indirectly affecting other species, including birds, reptiles, invertebrates, etc.). Competition with other

			ungulates is documented. The species is known to contribute to the spread of diseases and pathogens affecting both livestock and humans. It can also damage crops and compete with livestock. It can be a threat in relation to possible deer/vehicle collisions.
Conclusion of the risk assessment (overall risk)	low **moderate** high	low **medium** high	The axis deer represents a potential threat in the risk assessment area, given the ability to establish in the wild, the potential for spread, and the documented impact in other parts of the introduced range. Further warming of the climate due to climate change may increase impacts by increasing the amount of suitable habitat.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria			Yes	Yes	
Belgium			Yes	Yes	
Bulgaria			Yes	Yes	
Croatia	Yes	Yes	Yes	Yes	Yes
Cyprus			Yes	Yes	
Czech Republic	Yes		Yes	Yes	
Denmark			Yes	Yes	
Estonia				Yes	
Finland				Yes	
France	Yes		Yes	Yes	
Germany			Yes	Yes	
Greece			Yes	Yes	
Hungary			Yes	Yes	
Ireland	Yes		Yes	Yes	
Italy			Yes	Yes	
Latvia			Yes	Yes	
Lithuania			Yes	Yes	
Luxembourg			Yes	Yes	
Malta			Yes	Yes	
Netherlands			Yes	Yes	
Poland			Yes	Yes	
Portugal			Yes	Yes	
Romania			Yes	Yes	
Slovakia			Yes	Yes	
Slovenia	Yes		Yes	Yes	
Spain			Yes	Yes	
Sweden			Yes	Yes	
United Kingdom	Yes		Yes	Yes	

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	Yes		Yes	Yes	
Atlantic			Yes	Yes	
Black Sea			Yes	Yes	
Boreal			Yes	Yes	
Continental	Yes		Yes	Yes	
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian			Yes	Yes	
Steppic			Yes	Yes	

ANNEX I Scoring of Likelihoods of Events

(adapted from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[8]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area.

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[8] Not to be confused with “no impact”.

	with serious ecosystem effects			Widespread, severe, long-term, irreversible health effects.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	**Division**	**Group**	**Examples (i.e. relevant CICES “classes”)**
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*

		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)*
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*

	Water[9]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*
		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer*

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[9] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

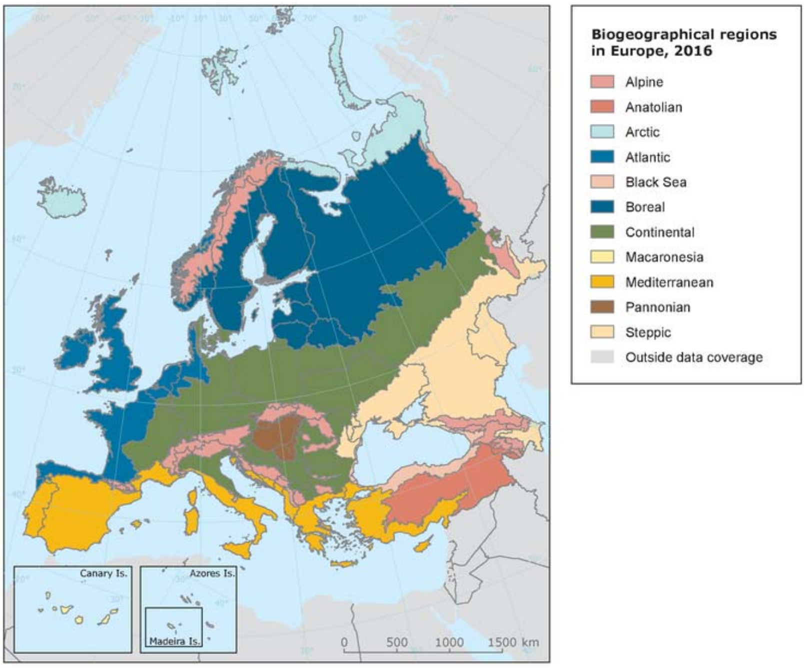
			strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

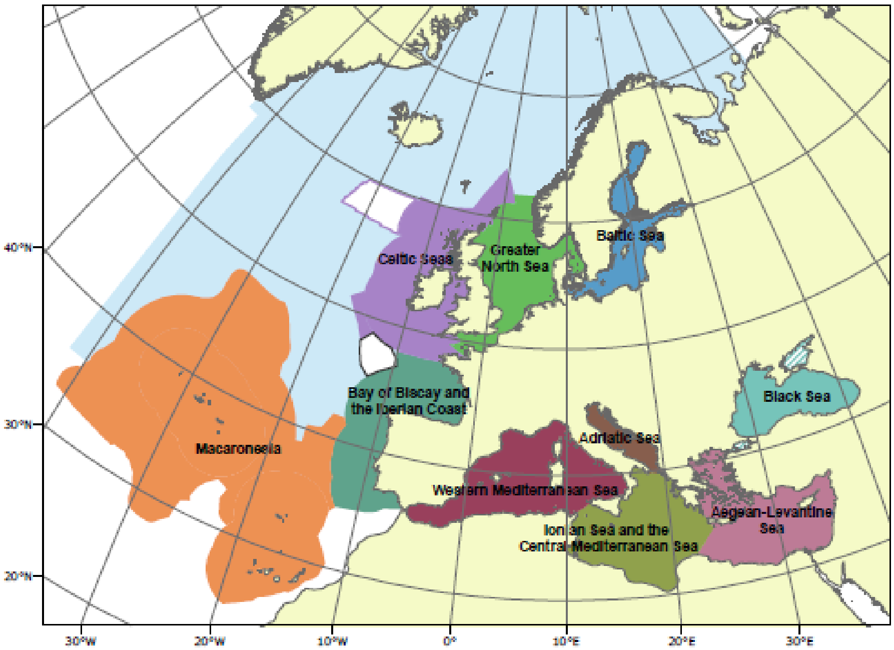
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

ANNEX VII Projection of climatic suitability for *Axis axis* establishment

Björn Beckmann, Riccardo Scalera, Beth Purse and Dan Chapman

30 October 2019

Aim

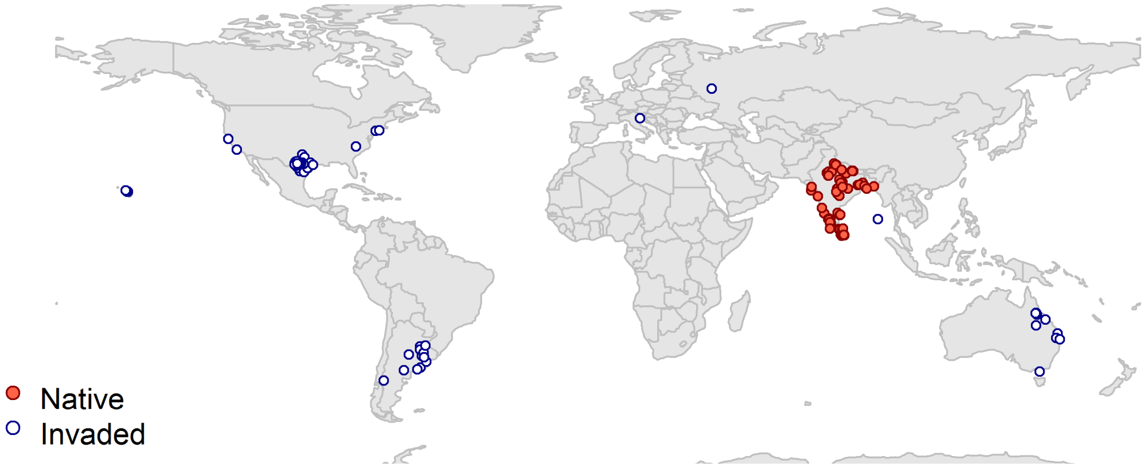
To project the suitability for potential establishment of *Axis axis* in Europe, under current and predicted future climatic conditions.

Data for modelling

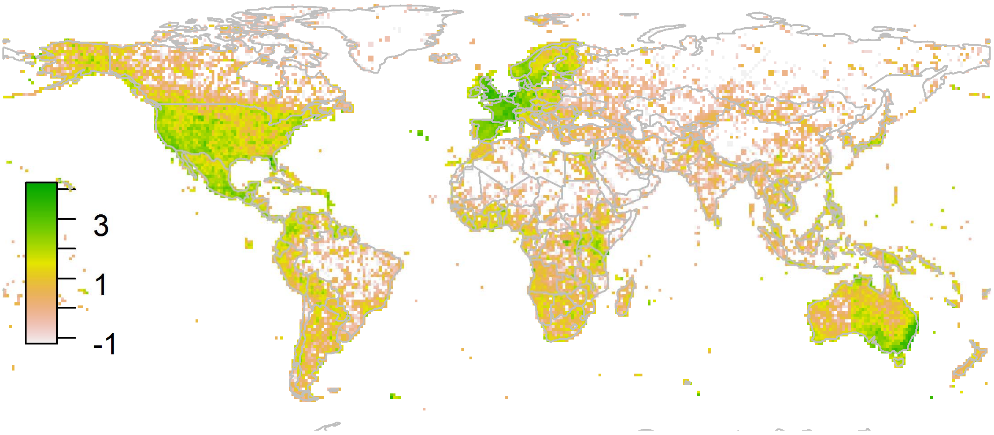
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (394 records), the Biodiversity Information Serving Our Nation database (BISON) (85 records), the Atlas of Living Australia (19 records), the Integrated Digitized Biocollections (iDigBio) (8 records), and a small number of additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils, captive records) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 156 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Mammalia records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Axis axis* and used in the modelling, showing native and invaded distributions. (b) The recording density of Mammalia on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.
Based on the biology of *Axis axis*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Annual precipitation (Bio12)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. There represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

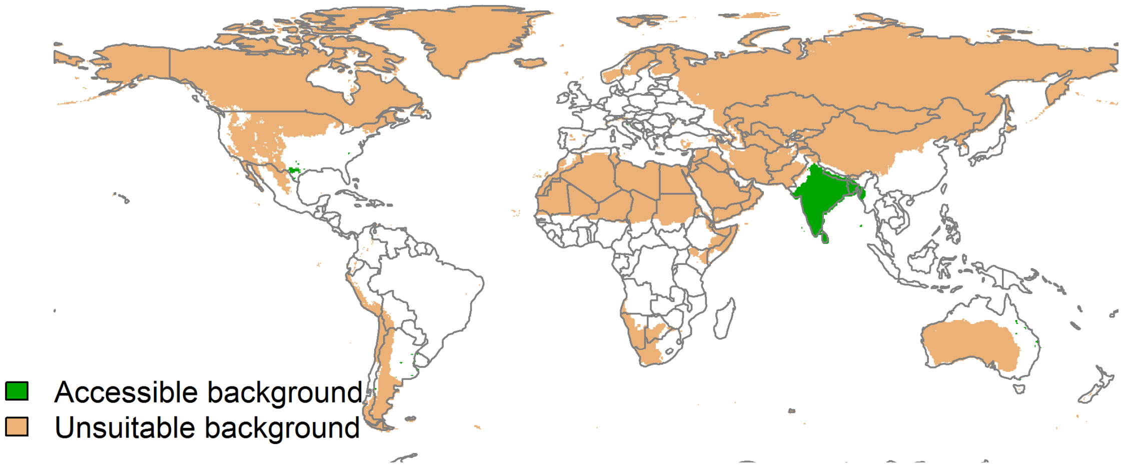
Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1 (Thuiller et al., 2019, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Axis axis* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 300km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Axis axis* at the spatial scale of the model:
    - Minimum temperature of the coldest month (Bio6) < -12
    - Mean temperature of the warmest quarter (Bio10) < 11
    - Annual precipitation (Bio12) < 6

Altogether, only 0.6% of occurrence grid cells were located in the unsuitable background region. Within the background region, 10 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Axis axis*. Samples were taken from a 300km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the background sample was much larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen’s Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa’s dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the ‘minROCdist’ method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Axis axis* was most strongly determined by Annual precipitation (Bio12), accounting for 45.9% of variation explained, followed by Minimum temperature of the coldest month (Bio6) (35.1%) and Mean temperature of the warmest quarter (Bio10) (19%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

					variable importance (%)		
Algorithm	AUC	Kappa	TSS	Used in the ensemble	Annual precipitation (Bio12)	Minimum temperature of the coldest month (Bio6)	Mean temperature of the warmest quarter (Bio10)
GLM	0.984	0.653	0.948	yes	47	35	18
GAM	0.982	0.668	0.949	yes	43	40	18
ANN	0.982	0.648	0.949	yes	44	37	19
GBM	0.977	0.671	0.946	no	45	33	22
MARS	0.981	0.660	0.950	yes	42	40	18
RF	0.978	0.639	0.938	yes	54	23	23
Maxent	0.976	0.675	0.943	no	46	32	22
Ensemble	**0.983**	**0.655**	**0.952**		**46**	**35**	**19**

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

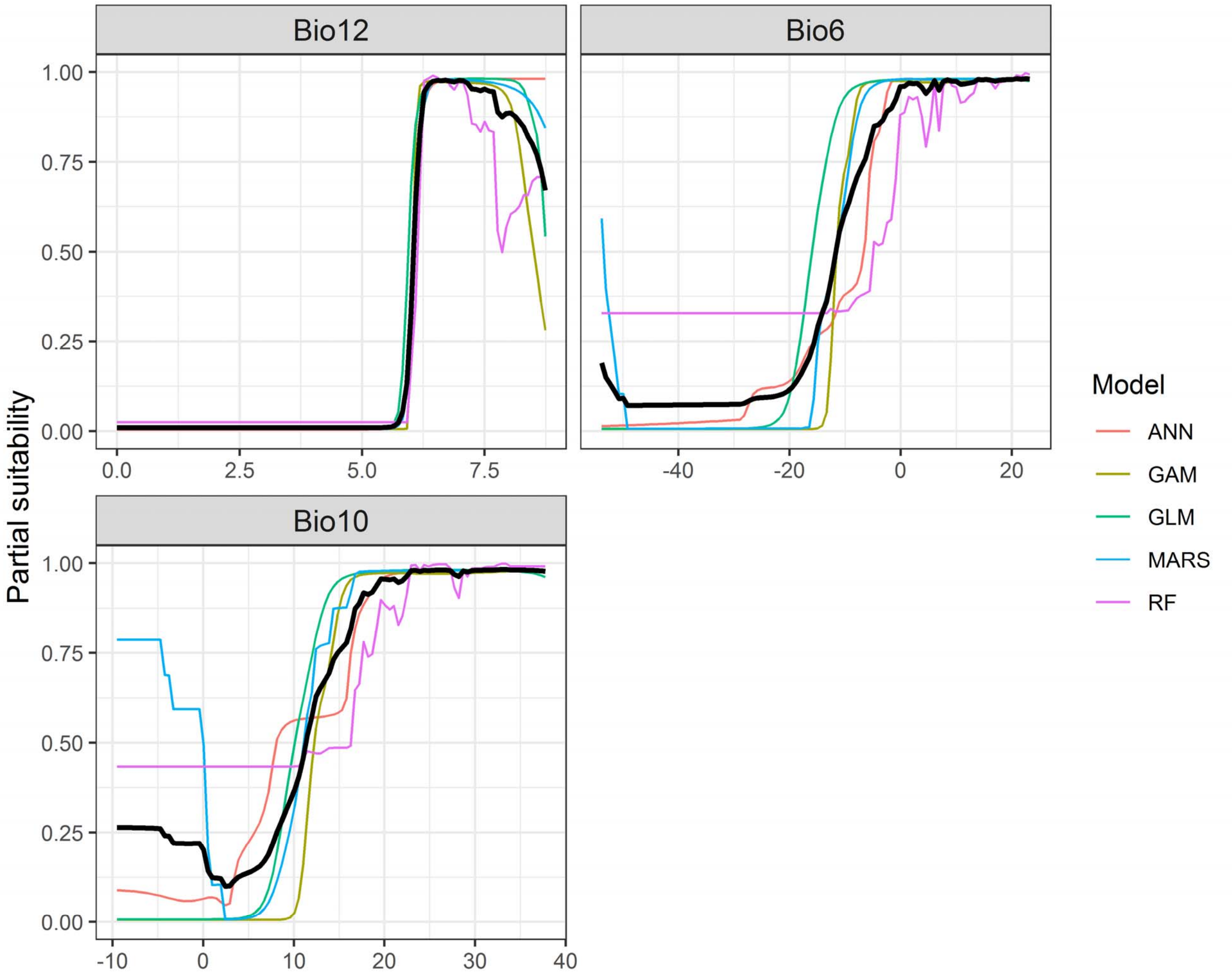
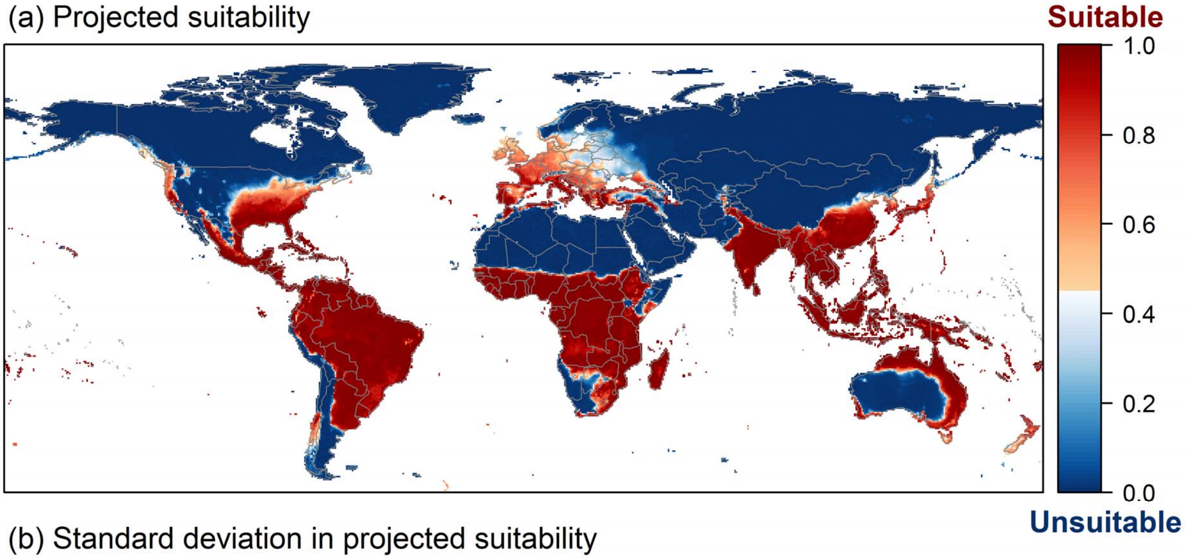


Figure 4. (a) Projected global suitability for *Axis axis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.45 may be suitable for the species. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

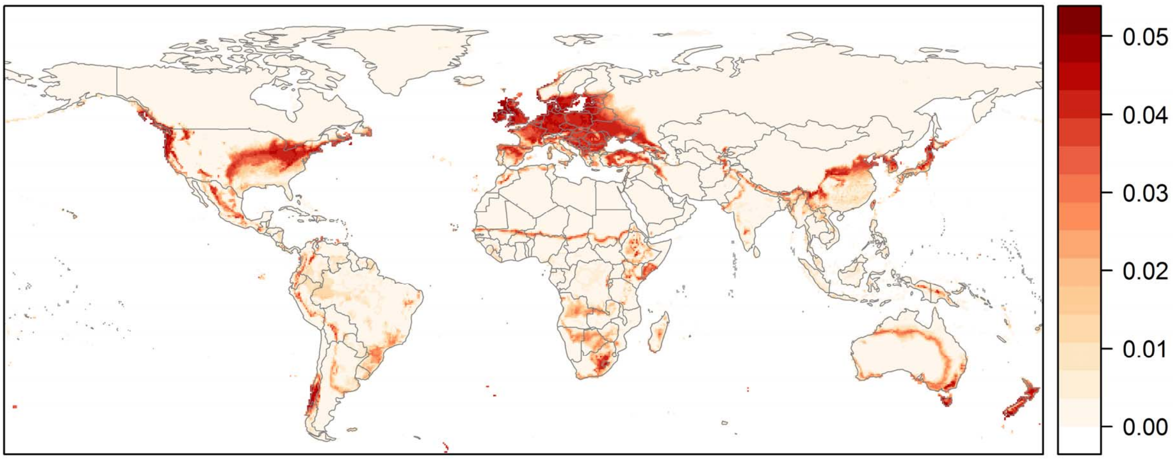
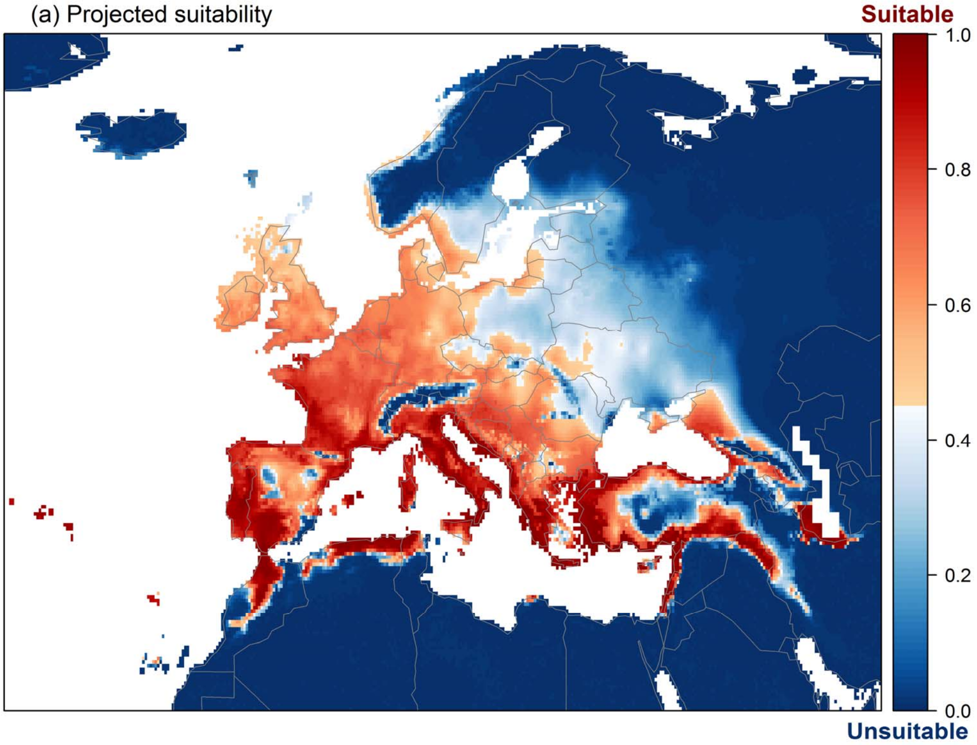


Figure 5. (a) Projected current suitability for *Axis axis* establishment in Europe and the Mediterranean region. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

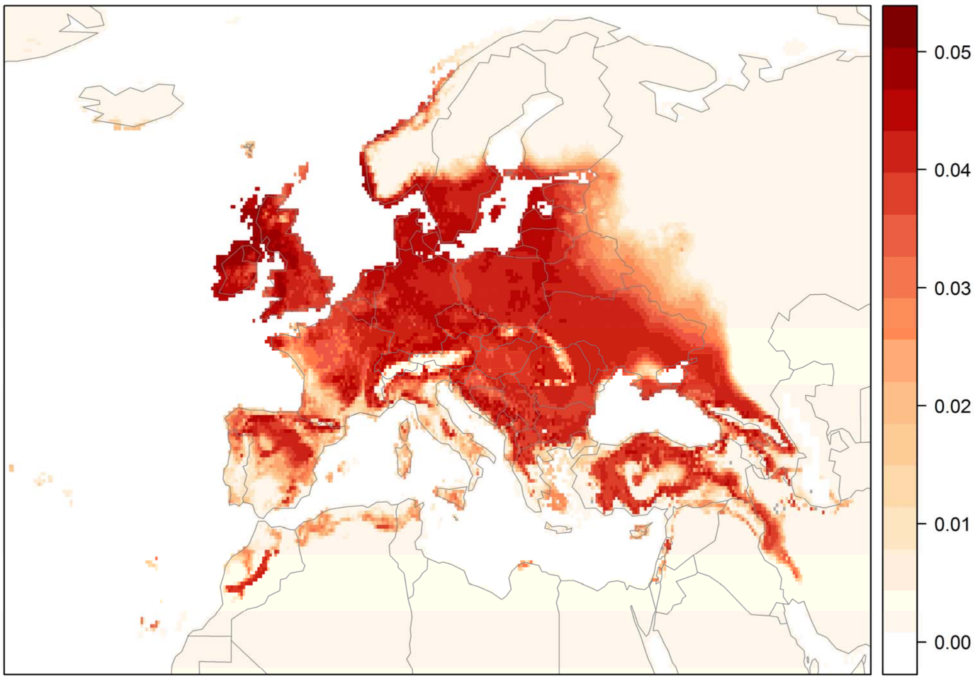


Figure 6. The most strongly limiting factors for *Axis axis* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

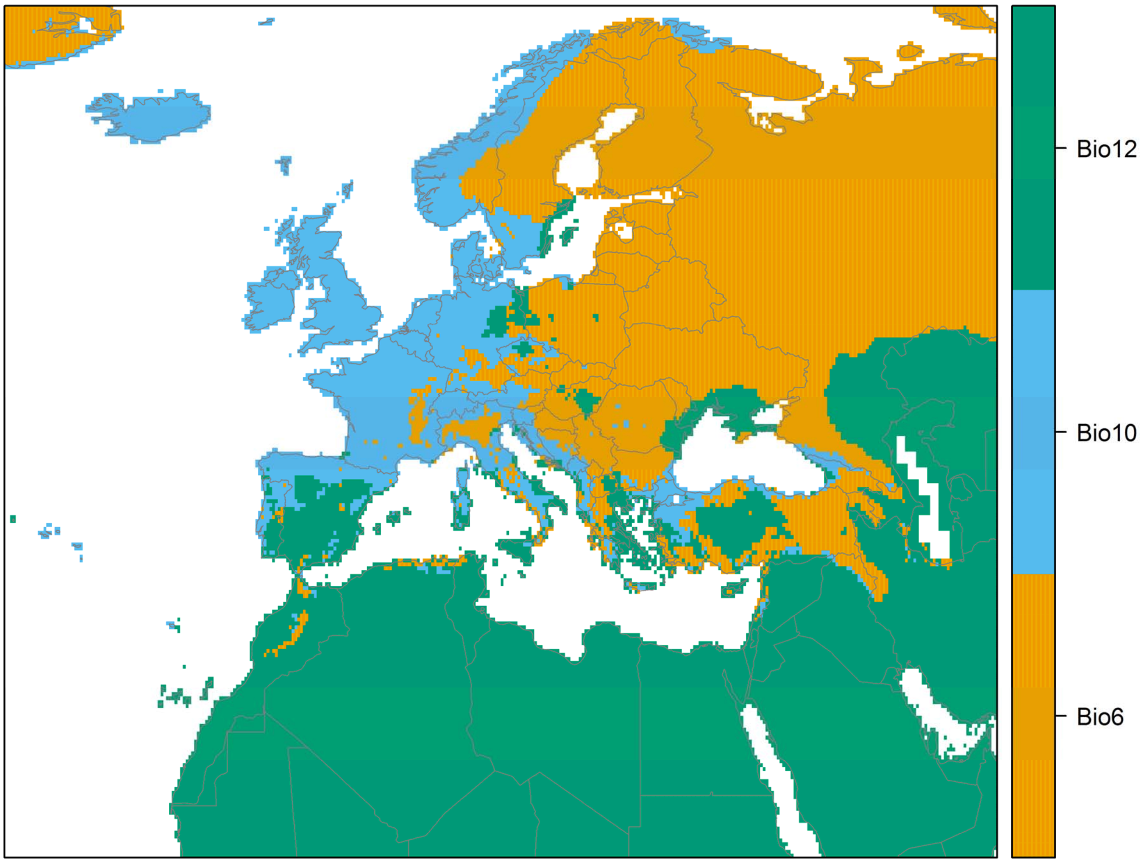
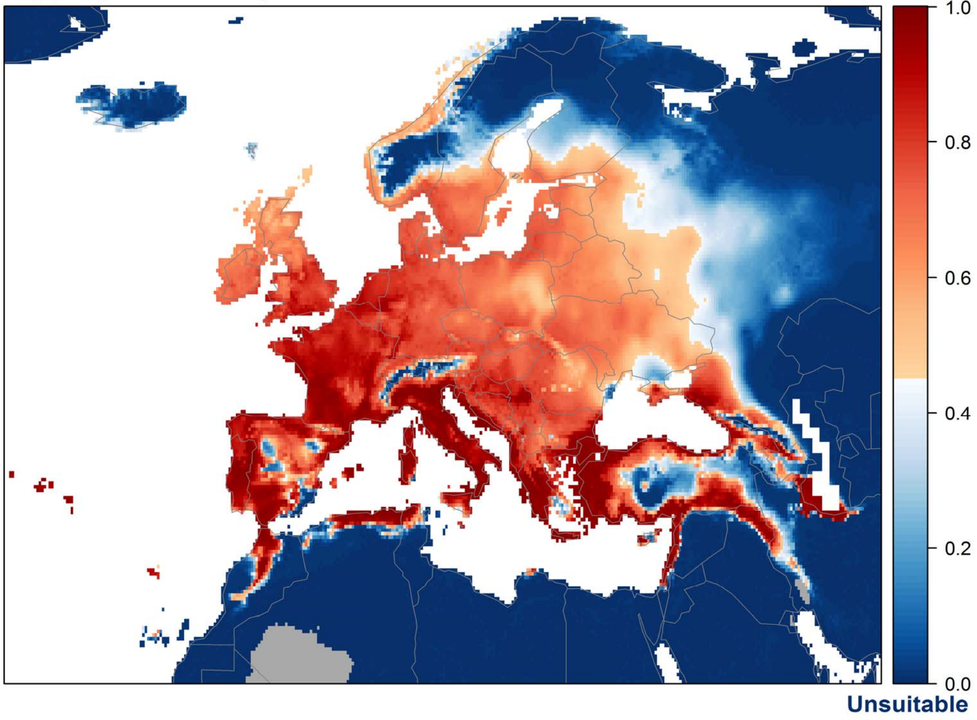
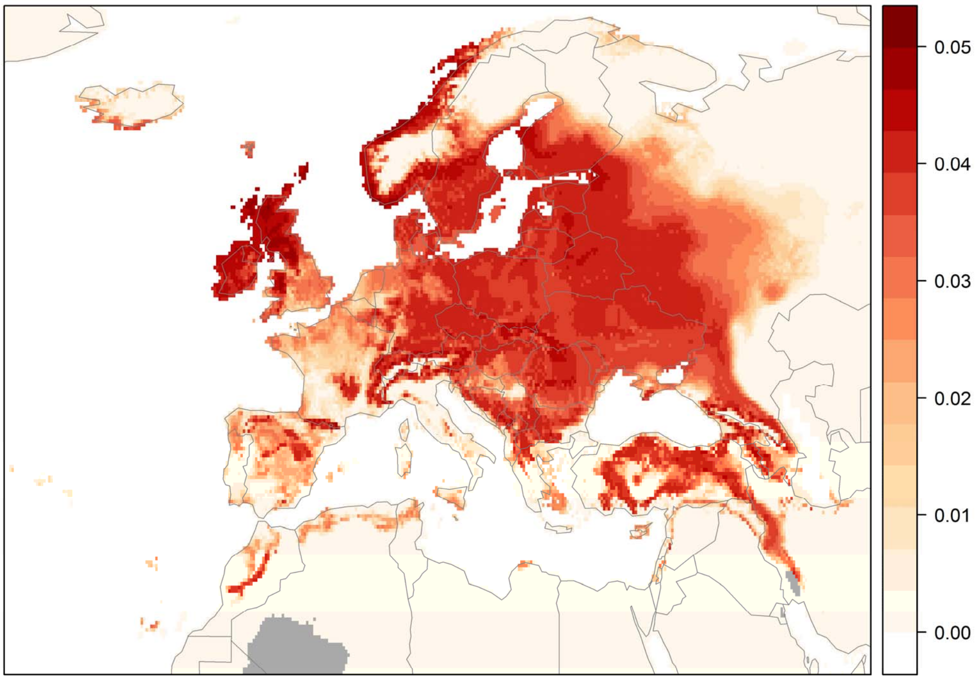


Figure 7. (a) Projected suitability for *Axis axis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

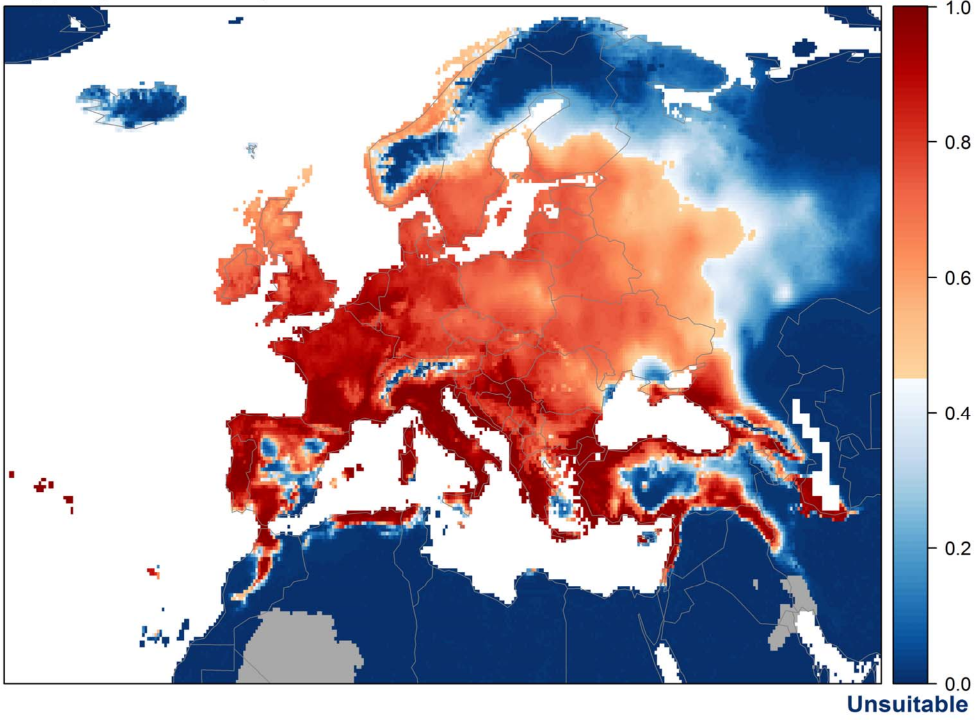


(a) Projected suitability

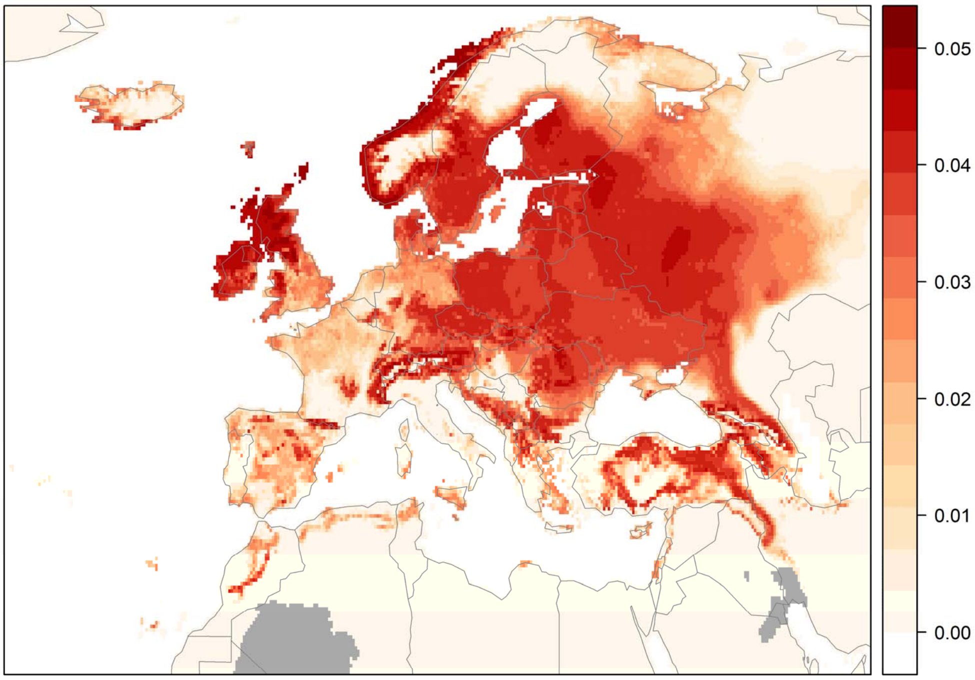


(b) Standard deviation in projected suitability

Figure 8. (a) Projected suitability for *Axis axis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



(a) Projected suitability



(b) Standard deviation in projected suitability

Figure 9. Variation in projected suitability for *Axis axis* establishment among Biogeographical regions of Europe (Bundesamt für Naturschutz (BfN), 2003). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

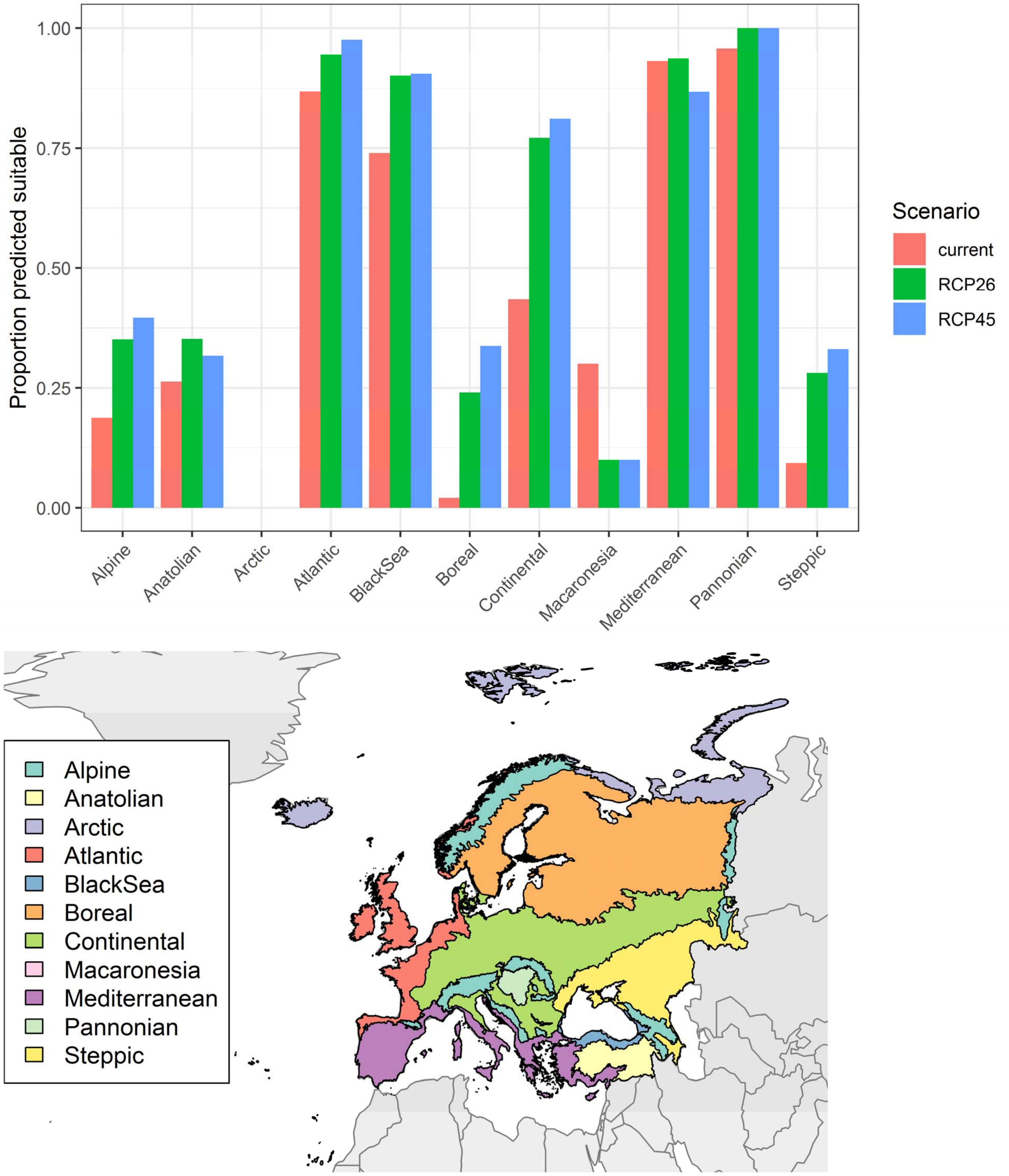
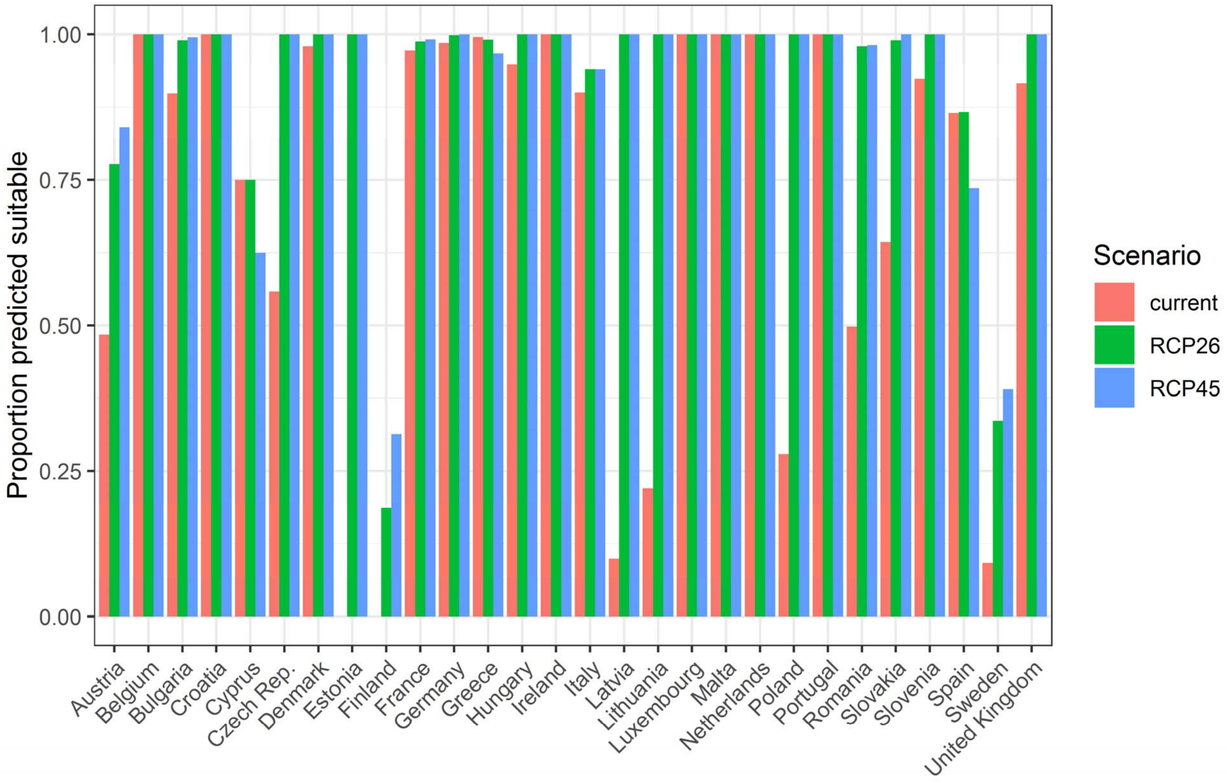


Figure 10. Variation in projected suitability for *Axis axis* establishment among European Union countries. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.



Caveats to the modelling

To remove spatial recording biases, the selection of the background sample was weighted by the density of Mammalia records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.
Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	*Axis axis*
Species (common name)	Axis deer, Chital deer, Spotted deer
Author(s)	Peter Robertson
Date Completed	23/04/2019
Reviewer	Riccardo Scalera

Summary [1]

Hihlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Axis deer have been widely introduced globally through escapes from ornamental collections, deer farms and releases to supplement hunting. The adoption and enforcement of appropriate legislation and codes of best practice to reduce the risks posed by these pathways should reduce the probability of further introductions in Europe. This can be assisted by raising public awareness of the impacts of non-native deer and invasive alien species in general. The effective reporting of new incursions, with records obtained from citizen science initiatives and through existing hunting networks, will reduce the costs of rapid response to new incursions.
There is little specific evidence available on the eradication of Axis deer, although this species has been removed from an island in Australia and attempts are underway in Hawaii. More widely, eradication programmes for other deer species and ungulates have relied on shooting and have been successful over large areas of up to 6,000km^2. Globally, the cost- effectiveness of ungulate eradications has been improved through the use of helicopter shooting, judas animals to locate remaining individuals, night shooting combined with infra-red cameras, fences and natural barriers to break larger areas into smaller components, and dogs to help locate animals. However, the use of these methods may be restricted in particular EU Member States and may attract opposition from the public and hunters on a case specific basis. Traps and toxins have been used as supporting methods in some eradication programmes. There are no toxins approved for use on deer in the EU.

The long-term management of deer populations is widespread in Europe, with established hunting traditions which value deer as a resource, and programmes to reduce impacts, such as local damage to crops and forestry, to acceptable levels. Current deer management practice mainly relies on shooting. The potential use of contraceptives as a deer management tool attracts frequent public interest, but currently such approaches rely on catch and inject methods. There are no examples of contraceptives being used to achieve eradication, and only limited examples are reported of their use on free-living populations. The use of fences, scaring methods and deterrents provide useful additional methods to reduce local damage.

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)[3]**	**Level of confidence[4]**
Methods to achieve prevention [5]			
Managing pathways	Axis deer, also known as Chital or Spotted deer, have been introduced to new areas, primarily as an ornamental species for collections, escapes from deer farms, or as an addition for hunting. The adoption and enforcement of appropriate legislation and codes of best practice to reduce the risks posed by these pathways should reduce the probability of further introductions		
Effective surveillance and reporting	Axis Deer are a highly visible species. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the species can become established.	Citizen-science species occurrence datasets are increasingly recognized as a valid tool for monitoring the occurrence and spread of invasive species across large spatial and temporal scale. They are dependent on citizen-scientists who collect and upload data, typically from 'opportunistic sampling' with no underlying scientific survey design which can limit the conclusions that can be drawn from these	

	Citizen science provides a useful route for the reporting of IAS. Deer are widely hunted and reports from the hunting community may be of particular value to detect new species in an area.	data. Most parts of north-west Europe have an extensive network of volunteer observers although this is less true of southern and especially eastern Europe. Nevertheless the focus on native species may lead to disregard the presence of non-native species, and consequently to a delay in detecting a new presence of this non-native species. However, this naturalist community also provides an opportunity for developing an effective surveillance system. Unstructured citizen-science data do not reliably allow the estimation of species abundance or population trends, yet in an early-warning scenario it is likely sufficient to know where a species is establishing, and these data limitations are thus of a lesser concern. For deer, reports from hunters can be an effective source of information on occurrence and distribution. Hunter records are often collated nationally to produce statistics on key species (Apollonio et al 2010) although the methods and formats used vary between Member States.	
Raising awareness	Raising public awareness of the risks posed by invasive alien species in general and Axis deer in particular. This can include the production of targeted publicity and identification material.	A number of information sources and sheets are available on-line which can assist with the production of new material to raise public awareness. Examples specific to Axis deer include: Texas http://www.tsusinvasives.org/home/database/Axis-axis Hawaii http://www.biisc.org/axis-deer/ South Africa http://www.invasives.org.za/component/k2/item/709-axis-deer-axis-axis	

		Australia https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/land-management/health-pests-weeds-diseases/pests/invasive-animals/restricted/chital-deer	
Methods to achieve eradication[6]			
Shooting to achieve eradication	Shooting is the most widely used and effective method to control deer (Côté et al. 2004). This underpins most current management of deer populations in Europe (Apollonio et al 2010) and is the most frequent method used for deer and other ungulate eradications worldwide (DIISE 2015). Deer are typically shot for sport with rifles by solitary hunters firing from a fixed position or by stalking the animals. Ungulate control programmes worldwide have used a wider variety of methods including the use of automatic weapons, night shooting, thermal imaging, use of fences and natural barriers to break up populations, dogs to locate animals, shooting	Axis deer are actively managed through shooting across most of their introduced range, including at least 13 states in the US, Argentina, Croatia, South Africa and Australia. The DIISE (2018) database includes details of 10 successful eradications of various deer species from islands. All of the cases that give details, refer to shooting as the primary method used. The largest reported successful deer eradications are of Mule deer *Odocoileus hemionus* and Elk *Cervus elaphus nelsoni* from the Santa Rosa Channel Island off the Californian coast, an area of 222km^2. Axis deer have also been successfully eradicated from a 45.7km^2 island off the coast of Victoria in Australia, although no details of costs or methods are available (Johnston 2008). There is an ongoing eradication attempt on Hawaii including the use of shooting from helicopters and infra-red cameras, although the eradication is hampered by local opposition and issues with land access (Hess et al 2015). No information on the costs of these deer eradications are available. However, Parkes (1990) and Carrion et al (2011) describe the costs and methods of large scale goat eradications from islands in some detail. These include	High. Shooting is widely used for controlling deer species, including Axis deer. It has been the primary method used to support deer eradication programmes.

	from vehicles and from helicopters.	successful removals from islands of up to 6000km^2 at a cost of around \$10,500,000 (\$1,750 per km^2) at 2011 prices, including the use of aerial hunting and Judas animals (Carrion et al 2011). The costs per unit area are known to vary in relation to the size of the area managed (Robertson et al 2017), the costs per unit area from smaller programmes will be substantially higher. The use of different firearms is heavily regulated and the details vary between member states. Many EU Member States specify the type of firearm, its calibre, number of cartridges and weight of ammunition together with the times of day and location where deer shooting is permitted. These are likely to restrict the nature of the weapon, the requirements for the operator and the times and locations where they may be used. Many Member States require those using shooting to control deer to undertake a specialist training programme to ensure they are competent and safe before issuing a license. Local authorities in the Member State must be consulted before their use. The use of firearms brings risks to health and safety which need to be managed. The use of lead projectiles has been restricted in some areas due to environmental concerns, although non-toxic alternatives are available. Although shooting is already widely used to control deer across Europe, its use in public places is likely to bring opposition and raise particular concerns. The use of single	

		shot rifles as used by sportsmen may be generally accepted, while the use of other methods such as automatic weapons, night shooting, thermal imaging, shooting from vehicles and helicopters are likely to all raise public concern, including opposition from hunters who may normally be prohibited from their use. As reported by Moriarty (2004) if animals such as deer were targeted for removal in response to an exotic disease outbreak, shooting from a helicopter would be one of the most effective means of implementing emergency control. But this would need to be weighed against the risk of disturbing and dispersing the deer population. Helicopter shooting is unlikely to be an economic option for general deer control.	
Hunting with dogs	Professional ground hunting with appropriately trained dogs may be used by hunters to take deer under conditions where conventional ground shooting would be difficult (Moriarty 2004).	Dogs which bring an animal to bay rather than attack would allow humane destruction of deer in circumstances which would otherwise make eradication impossible (Moriarty 2004). Deer hunting using trained dogs can cause much greater mortality and is less selective than shooting (Novak et al. 1991), while dogs can also cause greater disturbance which may decrease deer sighting rates (Godwin et al. 2013).	Medium This method has been used for the control of other deer, but no published evidence for its use with this species
Judas Animals	Judas animals are marked individuals that are used to identify the locations of other of their species to assist with control by locating remaining groups or individuals. Judas	The use of Judas animals is most effective on species which form social group or herds. Axis deer form large herds in their native range (Barrette 1991) although in Hawaii, Axis deer control using this method highlighted problems associated with the loose herd structure in this species. (Hess et al 2015). The current information on the use of	Medium. Judas animals have been used successfully to assist the eradication of other ungulates, but the

	animals are typically captured, radio-tagged, and then released to join a social group. This group can then be followed using the radio-transmitter to assist with lethal control, typically by shooting. By sparing the Judas animal, it can then be left to locate and join another social group and the process repeated. The ability of Judas goats to attract conspecifics has also been enhanced through the use of chemically induced oestrous (Campbell et al 2007), to lure otherwise wary males. Originally used to improve the efficiency of feral goat control (Taylor and Katahira 1988, Campbell and Donlan 2005), these approaches have now been used on a range of social mammals including deer (Crouchley et al 2011) to improve the efficiency of eradication programmes.	this method on Axis deer is equivocal although this is based on very limited experience. No costs for the use of Judas deer are available. However, Campbell and Donlan (2005) describe large improvements in the cost-effectiveness of goat eradications since the application of this approach. This method has been predominantly used on uninhabited islands, although it is now also widely used in Scandinavia to assist the control of raccoon dogs. Little information is available on the public reaction to this approach. However, an adverse public reaction remains a possibility.	only reported use on Axis deer was equivocal.
Traps	A wide variety of traps and nets have been used to catch deer.	There are no accounts of deer eradication programmes based on the use of traps as the primary control method.	Medium – traps have been used as a

	However, these are typically used for live capture rather than directly as a control method. A variety of designs such as drop-nets (Ramsey 1968, Conner 1987), Stephenson traps, clover traps, rocket nets and darting (Haulton 2001) are all described in the literature, with information on their success and associated rates of injury. Deer may also be captured in corrals. Mitchell (2016) describes a large corral trap design for use on this species. Advantages and disadvantages of trapping (as a control technique that can be used in conjunction with other techniques, such as fencing) are summarised by Mitchell (2015) along with advice for an effective implementation of the method.	However, Crouchley et al (2011) used traps as a subsidiary method during the eradication of red deer from an island of New Zealand. Live capture is needed for the Judas animal method, and may form a useful part of eradication programmes based on this method. The live capture of deer risks stress and injury to the animals, requiring experienced personnel to reduce the risks. Conner (1987) estimates mortality associated with drop traps to be between 1 and 7% within a week of capture. Haulton (2001) reports between 2-20% trap related mortality. While trapping may remove deer, it is likely that remaining trap-shy individuals would still need to be removed by other means, for example shooting is likely to be required at some stage if complete eradication is the objective (Moriarty 2004) Mitchell (2016) describes the design and initial testing of a large corral trap (200m circumference) to capture multiple Axis deer in Queensland Australia. Baited with alfalfa, this has attracted up to 26 animals to enter the corral at one time, but only a small number of captures were described as this was largely a testing project rather than practical implementation at this stage	minor method to support deer eradication programmes and the live capture of Judas animals. Although designs of corral traps were tested for use on this species, no descriptions of their practical use for management of Axis deer were found.
Toxins	Toxins have not been widely used for the management of deer. The toxin 1080 (sodium monofluoroacetate) has been	No toxins are currently approved for use on deer in the EU. The use of toxins to manage deer in the EU could be expected to raise considerable public opposition and can be	Medium – although toxins have been used for deer management, this

	incorporated into a gel and smeared on leaves as a deer toxin in New Zealand (Bacheler and Challies 1988). DIISE (2018) includes two New Zealand reports applying aerially based Brodifacoum baits as a secondary method to achieve deer eradication, but shooting forming the primary method in each case. The New Zealand experience suggests that 1080 was expensive when applied over large areas, but it was used successfully to control localised populations of deer that cannot be killed by other means (Moriarty 2004).	criticised for its likely impact on non-target species (Moriarty 2004)	has typically been to support other methods such as shooting. No data is available related to their use on Axis deer.
Surveillance during eradication	Monitoring the reduction in population size and identifying the number of remaining animals is an important component of an eradication programme. Many programmes have used declines in catch, signs or sightings per unit effort to	The use of DNA to estimate the size of any remaining population during eradication offers improvements in cost-effectiveness. However, animals need to be sufficiently genetically distinct for the method to be effective. Small populations derived from a limited founder stock may be too similar for the method to be effective (Crouchley et al 2011).	Medium. This method has not been widely used to assess its effectiveness, nor applied to Axis deer.

	assess progress. More recently, programmes have collected DNA from culled animals and from faeces (pellet) found in the field to estimate the number of remaining animals in a population, (Nugent et al 2005, Crouchley et al (2011)	Pellet counts are widely used to monitor ungulates but rely on the assumption that pellets of different species are correctly identified in the field. However recent eDNA studies revealed high rates of misidentification in diverse European ungulate communities monitored through pellet counts (Spitzer et al. 2019)	
Methods to achieve management [7]	All of the methods described to support eradication can also be used to manage existing deer populations. In Australia, the Queensland Department of Agriculture, Fisheries and Forestry (2013) provide a simple review of methods for ongoing deer management, including *Axis*		
Shooting	Shooting is by far the most common method of managing deer populations. Shooting is the main control method used for the long-term management of Axis throughout its introduced range. For example, in Argentina Axis the deer population on a nature reserve were managed mostly	As emphasized by Gürtler et al. (2018) the most successful control strategy for axis deer is ground shooting including commercial harvesting, state-funded culling and sport hunting or a combination thereof. The principle challenge faced using hunting is usually land ownership and deer distribution.	High. Shooting is widely used for the control of deer species, including Axis deer in a range of countries

	through controlled still shooting from watchtowers, and partly though firearm shooting from vehicles (Gürtler et al. 2018). Advantages and disadvantages of both aerial shooting and ground hunting are summarised by Mitchell (2015) along with advice for an effective implementation of the method.		
Contraceptives	A wide variety of contraceptive agents have been considered for use on deer, including surgical implants, injectable contraceptives and orally delivered products. These can work through a number of different mechanisms, including supplementing or replacing natural hormones; inducing an immune response to elements of the reproductive system, such as sperm, egg surface proteins or particular hormones involved in reproduction. Two products have received	The use of contraceptives is of proven effectiveness for individual animals, but to reduce a population a significant proportion of the reproductive animals must be treated. This raises complex issues of scale, cost and practicality that have limited the widespread use of these products to date. There are currently few examples of the successful use of wildlife contraception beyond small, closed populations such as on islands, in suburbia or in fenced enclosures such as parks (IUCN 2017). There are also no examples of the use of contraceptives to eradicate an established population of deer or to reduce deer abundance over any significant area. Beyond surgical implants, currently the only commercially available immune-contraceptive products are PZP and GonaCon although their use if limited to particular species	Medium. Despite the large volume of research on wildlife contraception, there are currently few examples of its practical use to control deer or examples of its use to achieve eradication

	particular attention. Injectable Porcine Zona Pallucida (PZP) raises an auto-immune response in the female to egg surface proteins, preventing fertilisation. GonaCon is another injectable product that raises an auto-immune response to GnRH, the hormone that provides high level regulation of other sex hormones; suppression of GnRH maintains the animal in a non-breeding or juvenile condition. The products and their potential for use have been reviewed by Fagerstone et al (2010) and in relation to the management of alien invasive species in Europe (IUCN 2017). The UK Deer Initiative have also reviewed the prospects of deer control using contraceptives in the UK.	and this varies between countries. Neither is currently specifically licensed for use on Axis deer. There may be a variety of welfare consequences for the treated animal dependent on the product chosen. For example, females treated with PZP may undergo prolonged oestrus together with its associated behaviours. This can lead to stress and loss of body condition in the females from male harassment. Products such as GonaCon may interfere with the development of secondary sexual characters in males, such as antlers (Killian et al 2005), with welfare implications. These can be avoided by the careful selection of products and their targeted delivery to only one sex, although these can increase the cost and limit the practicality of their use. The injection of immuno-contraceptive products may be associated with the formation of abscesses at the injection site (Miller et al 2008). The welfare implications of these products are reviewed by IUCN (2017). Proposals to use contraceptives and avoid lethal control are often welcomed by the public and many stakeholders. Proposals for the use of contraceptives often receive opposition from deer hunting interests who are concerned about contamination of deer products from animals that they might subsequently hunt, the perceived impracticality of contraception for use at a large scale, and the lack of recognition for the traditional role of hunters in managing deer populations.	

		The practical costs of contraceptive use are currently high as they are typically based on the use of small numbers of implants or injectable products. These can involve the capture and surgical treatment of individual animals with associated costs and risks to animal welfare. If orally delivered contraceptive are developed, species specific delivery systems perfected and the appropriate licenses gained, then the costs may be dramatically reduced in future. As reported by Gürtler et al. (2018) fertility reduction (contraception) and non-lethal removal have only played marginal roles for deer management, and was considered unsuitable for most situations in a review discussing the efficacy and cost-efficiency of deer management methods in Australia (Davis et al. 2016).	
Fencing, Deterrence and Resource Protection	Localised damage caused by deer can be managed by the use of deer proof fences. These are widely used in commercial forestry to protect young plantations. However, they bring problems of reduced access for other purposes, reducing their use to protect agricultural crops or other resources. Individual trees can be protected during early growth through the use of deer guards.	Designs of deer proof fencing, both wire and electric, scaring methods and repellents are available on-line and from commercial companies. Useful sites include: The Internet Centre for Wildlife Damage Management: **http://icwdm.org/handbook/mammals/Deer.asp** As emphasized by Gürtler et al. (2018) the most successful control strategies for axis deer to date are believed to be fencing and ground shooting including commercial harvesting, state-funded culling and sport hunting or a combination thereof.	High. Methods such as fencing are widely used to reduce local deer impacts

	A wide variety of scaring and deterrent products have been proposed to limit local damage (Côté et al. 2004). The effectiveness of these methods is highly variable and local trails are required to assess the usefulness of each for a specific purpose. Advantages and disadvantages of exclusion fencing and of deterrents are summarised by Mitchell (2015) along with advice for an effective implementation of the method.	As reported in Hawaii (Hess 2008) and Texas (Anderson, 1999), axis deer have been observed to jump fences about 2.5 m tall. Building fences high enough to totally exclude deer is expensive and difficult. Therefore, supplementary control efforts are often needed within areas enclosed by lower fences.	
Biological controls	Predation by wild dogs, wild boar, foxes and eagles is believed to limit deer populations in some areas (Moriarty 2004, Gürtler et al 2018). But the role of predators in controlling ungulate populations remains uncertain, at least in some systems (Côté et al. 2004).	Young animals would be the main targets. There are no other biological controls that appear useful as a control agent for deer (Moriarty 2004). Release of biological control agents for axis deer seems of little relevance for the EU. Two male leopards (*Panthera pardus*) were introduced in the Andaman Islands on this purpose, but no results are reported (Long 2003, Sivakumar 2003). Gürtler et al (2018) found an inverse relationship between wild boar control and Axis deer abundance during the course of a long-term control operation in Argentina, and speculated that predation of deer fawns by boar may provide a mechanism for this effect	Low. Although wild deer are impacted by predators, there is little evidence of the successful use of this approach to manage deer populations

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; this is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: red-vented bulbul (*Pycnonotus cafer*)

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Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

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Date of completion: 31/10/2019

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:

The red-vented bulbul belongs to the class Aves, order Passeriformes, family Pycnonotidae. The scientific name for the red-vented bulbul is *Pycnonotus cafer*, and the author is Linnaeus, 1766. Earlier names include *Turdus cafer* (Linnaeus, 1766), *Molpastes haemorrhous* (J.F. Gmelin, 1789), *Pycnonotus pygaeus* (Sharpe, 1881) and *Molpastes cafer* (Baker, 1930). Other names for the species in English and in other languages include common bulbul or sooty-headed bulbul (Thibault, 2018a), bulbul ventirrojo (ES), Bulbul à ventre rouge (FR), Rußbülbül (DE), Kala buulbuul (NL), roodbuikbuulbuul (NL) and Bilbil czerwonoplamy (PL).

The genus *Pycnonotus* comprises 48 species (Delacour, 1943; Dickinson and Dekker, 2002; Gill and Donsker 2018).

Pycnonotus cafer comprises eight subspecies (Dickinson et al., 2002):

- Central Indian red-vented bulbul (*P. c. humayuni* Deignan, 1951), found in south-eastern Pakistan, north-western and north-central India;
- Punjab red-vented bulbul (*P. c. intermedius* Blyth, 1846), in Kashmir, Kohat down to the Salt Range and along the western Himalayas to Kumaon, originally described as a separate species;
- *P. c. bengalensis* (Blyth, 1845), in central and eastern Himalayas from Nepal to Assam, north-eastern India and Bangladesh, originally described as a separate species;
- *P. c. stanfordi* (Deignan, 1949), in northern Burma and south-western China;
- *P. c. melanchimus* (Deignan, 1949), in south-central Burma and northern Thailand;
- *P. c. wetmorei* (Deignan, 1960), in eastern India;
- *P. c. cafer* (Linnaeus, 1766), in southern India;
- *P. c. haemorrhousus* (Gmelin, 1789), in Sri Lanka (Dickinson et al. 2002).

In its native range, *P. c. humayuni* is known to hybridize with *Pycnonotus leucogenys* (Gray, 1835). These hybrids were once described as subspecies *magrathi* (Sibley & Short, 1959). Hybridisation with *Pycnonotus leucogenys* has also been observed in the United Arab Emirates (Khan, 1993) and Bahrain (Khamis, 2010). The offspring of hybrids in Bahrain is believed to be sterile (Khamis, 2010). In eastern Myanmar, there is some natural hybridization with *Pycnonotus aurigaster* (Sharpe, 1909; Rasmussen & Anderton, 2005). Hybridisation with *Pycnonotus leucotis* (Gould, 1836) has been observed in Kuwait (Gregory, 2005), Qatar (Nation et al., 1997) and Iran (Azin et al., 2008). Hybridisation with *Pycnonotus xanthopygos* (Hemprich & Ehrenberg, 1833) has been observed in the United Arab Emirates (Khan, 1993).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

Pycnonotus cafer has brown feathering and a black crest on its head, neck and throat (Thibault, 2018a; Pratt et al., 1987). Its sub-caudal feathers have a bright crimson color, hence the species' name (Berger, 1981; Zia et al., 2014). It has a pale grayish white lower belly and rump which is highly visible in flight, and a long tail with a white tip (Zia et al., 2014). The tips of the back and breast feathers are white and look like scales (Thibault, 2018a). The tail is black with a narrow white tip (Thibault, 2018a). The red-vented bulbul measures about 21 cm in length (Berger, 1972) and its weight can vary from 26 to 45 grams (Long, 1981). Males can measure up to 23 cm in length, slightly larger than females, which is the only sexual dimorphism in the red-vented bulbul (Stuart & Stuart, 1999). Juveniles look like adults but with paler feathering and brownish edging on the feathers (Thibault, 2018a). It may live for up to 11 years (Walker, 2008).

The red-whiskered bulbul (*Pycnonotus jocosus*) or crested bulbul, has been introduced to many regions of the world, including in Europe (Spain). The red-whiskered bulbul has an erect black/dark brown crest, a dark brown/black head with prominent white cheek patches and red whiskers below each eye. It is widely kept as a cage bird, escaped and established in many places, including Australia, Madagascar, Hawaï, Japan, the Seychelles, the USA and Spain. It is a pest of agriculture and gardens, feeding on fruits, vegetables, flower buds and insects. Furthermore, it is known to have environmental impacts through the dispersal of seeds of invasive plants, interspecific competition and predation on geckos and invertebrates (Hawaii Invasive Species Council, 2017; Cottrell, 2017).

In Europe, both the red-vented bulbul and the red-whiskered bulbul are established sympatrically in Valencia, Spain (Lever, 2015; Dyer et al., 2017), so it is possible that one species could be confused for the other in this region. Another alien species physically resembling the red-vented bulbul is the sooty-headed bulbul (*Pycnonotus aurigaster*), mostly because of its red vent and black head. However, the breast and belly of *Pycnonotus aurigaster* is lighter and only birds of the *chrysorrhoides* group have a red vent (Fishpool & Tobias, 2019). The sooty-headed bulbul is one of the most abundant and widespread native bulbuls on Java and Bali. It has been introduced to Sumatra, Singapore and Borneo where its alien populations have been expanding since the 1980s. It is suggested that this spread can be attributed to escaped birds from captivity (Phillipps & Phillipps, 2011). However, the species currently has no known alien populations in Europe. The only other bulbul with a known alien population is the yellow-vented bulbul (*Pycnonotus goiavier*), which is native to the Malay Peninsula, Borneo, Thailand and the Philippines, and has an alien population on Sulawesi (Lever, 2005). However, the yellow-vented bulbul does not possess similar physical characteristics to the red-vented bulbul.

The only native species that somewhat resembles the red-vented bulbul and that therefore could be misidentified as such is the common bulbul (*P. barbatus*). This species is one of the commonest birds in Africa but is very rare in the wild in the risk assessment area. In recent years, common bulbul has bred in Tarifa (Cádiz, Andalusia, Spain) in an area that is potentially suitable for the red-vented bulbul as well. Birds are still present in the area, but only one pair has been known to breed and its status in Europe is currently tenuous (personal communication K. Bensusan, 22/10/2019). In 2013, two adult common bulbuls were observed feeding a young, which represented the first breeding record for the species in Europe (van den Berg & Haas, 2013).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

No other risk assessments for the species were found. However, as a well-known invader listed on the IUCN/ISSG list of 100th of the worst invasive species (Lowe et al. 2004), the species was included in several scoring exercises of invasive bird impacts globally. The results of these exercises are discussed in Qu. 5.1. An assessment of the impacts of the red-vented bulbul, undertaken using the Environmental Impact Classification for Alien Taxa (EICAT) classified the species as having Moderate (MO) impacts[2] through competition with native birds and by spreading the seeds of alien plants (Evans et al., 2016). The red-vented bulbul is also listed in the DAISIE database, however without any specific assessment.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is

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[2] EICAT categories are, from low to high impact: Minimal Concern (MC), Minor (MN), Moderate (MO), Major (MR), Massive (MV). MO impacts cause declines in populations of native species but do not cause native species extinctions.

naturally occurring

- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:

Pycnonotus cafer is native to the Indian Subcontinent, Southeast Asia, and Malay Peninsula (Long, 1981). It occurs naturally from Eastern Pakistan to southern China and Vietnam, and from Northern India to Sri Lanka. The species is linked to equatorial climate according to the Kopper-Geigen classification (Kottek et al., 2006) and can live in diverse habitat types. It is found in open areas, dry scrub, plains, cropland, natural forests as well as plantations and even urban areas (Vander Velde, 2002). It is in fact preferentially present in anthropogenic environments (urban areas, gardens, parks, farms), savannah areas, shrub vegetation, and more rarely on the edge of the forest (Vander Velde, 2002). In India, the species is used for bulbul fighting, a traditional yet recently prohibited custom at harvest festivals (Ratnagar, 2015).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

According to Thibault et al. (2018c), *Pycnonotus cafer* was introduced into 19 countries and established in 17 of them. The species is now present on at least 37 islands and seven continental locations (Thibault et al., 2018c). In order of first detection these are: Fiji, Australia (extinct), Tonga, the Independent State of Samoa, New Zealand (eradicated), American Samoa, USA (Hawaii, Texas), Qatar, United Arab Emirates, French Polynesia, Saudi Arabia, Kuwait, New Caledonia, Bahrain, Oman, Spain (Fuerteventura - Canary Islands), the Marshall Islands and Iran (Thibault et al. 2018c).

The first record of the red-vented bulbul outside of its native range was in Fiji in 1903 (Parham, 1955). It was probably brought there by Indian immigrants in the early 1900s (Watling, 1978), as it was widely used in bird fights in India because of its aggressive behaviour (Ali & Ripley, 1971). The bird has been recorded 6100 times in eight Pacific archipelagos since this first detection (Thibault 2018a).

The red-vented bulbul was detected in Melbourne and Sydney, Australia in 1918 and again in Melbourne in 1942 (Lendon, 1952; Watling, 1978; Dyer et al., 2017). The subspecies *P. c. bengalensis* was found in Melbourne in 1982 (Dyer et al., 2017). There are no records of the species since then. It is considered to be eradicated in Australia (http://www.issg.org/database).

The red-vented bulbul was deliberately introduced in the 1940s in Tonga to control unwanted insects (Watling, 1978). The red-vented bulbul probably reached the Independent State of Samoa in the early 1950's, and has since spread to several islands in the archipelago (Dhondt, 1977). The first observation of red-vented bulbul in Auckland, New Zealand was in 1952, but the species was eradicated in 1955 (Turbott, 1956; Watling, 1978). The red-vented bulbul was introduced in American Samoa in the late 1950s and quickly became established (Freifeld, 1999). First observations for Hawaï (USA) date from 1966. In French Polynesia, the red-vented bulbul was first noticed in the residential area of Papeete in 1979. They are now common on Tahiti on elevations of up to 1000 m, possibly having negative interactions with the Tahiti monarch (*Pomarea nigra*) (Blanvillain et al., 2003). The species was intentionally released in Nouméa (New Caledonia) around 1983 by bird dealers to avoid prosecution (Thibault et al., 2018c). The red-vented bulbul was first sighted on the Marshall Islands in 2000 near the major commercial dock of Majuro and it has been known to hitchhike on ships in other areas (Vander Velde, 2002). In 2002, there were already several breeding populations (Vander Velde, 2002). Several individuals were seen by Christina Sylvester on the Kwajalein Atoll in November 2018 (http://www.underwaterkwaj.com/land/bird-kwaj/bird-kwaj.htm, visited on 07/06/2019).

In the Middle East, the red-vented bulbul was first detected in Qatar in 1971, the first record for the bird around the Persian Gulf (Nation et al., 1997). It has been reported 3080 times in five countries around the Persian Gulf since this first detection. The red-vented bulbul was first detected in the United Arab Emirates in 1974 (Pedersen & Aspinall, 2009), in Saudi Arabia in the 1980s (J. Babington, pers. comm.). In Kuwait, the red-vented bulbul was first observed in 1981, and it is currently scarce, with a declining range (Gregory, 2005). According to Khamis (2010), the red-vented bulbul was first recorded in Bahrain in 1986, likely following an escape. At present, the bird maintains a self-sustaining population here. The first observation in Oman dates from 1987 and the the red-vented bulbul is now a common bird there (Thibault et al., 2018a). In Iran, the red-vented bulbul was first recorded in 2007 when 10-12 individuals were observed in the east of Kish Island, Hormozgan Province (Azin et al., 2008).

The red-vented bulbul was first observed in Houston (Texas, USA) near the end of the 1990's. At least 14 sightings were reported between May 1999 and March 2004. These sightings have been estimated to represent 32 birds at 10 sites in Houston (Eubanks et al., 2006).

A population is also established on Fuerteventura (Canary Islands, Spain) with the first sightings in the late nineties. By 2017, the species had spread over the entire island (SEO/Birdlife 2017; Nowakowski and Dulisz 2019).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a): Mediterranean, Atlantic

In the Netherlands, there have been multiple sightings of escaped individuals in the wild since 2001 (waarneming.nl). In Belgium, there was one observation of an escape in a natural area in a military Domain in 2005 (Brecht, Antwerp province). There are probably many more unreported incidental records of escaped birds across the risk assessment area. In Spain, there are some observations in Málaga and Torremolinos (personal communication A. Paterson).

Response (6b): Mediterranean

In Valencia (Spain), the species seems to be established, but no research has targeted this species yet. Breeding was observed in 2017 in gardens of urbanized areas surrounding the city. The distribution is not big, but they seem not to be uncommon. Here, the species is sympatric with the well-established red-whiskered bulbul (*P. jocosus*). (https://www.miteco.gob.es/es/biodiversidad/temas/inventarios-nacionales/; personal communication C. Gutiérrez-Expósito, 12/12/2018).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Mediterranean

According to the species distribution model (SDM, Annex VII), the red-vented bulbul could potentially establish in the Mediterranean biogeographical region. However, only around 2% of this bioregion is predicted to be suitable under current climatic conditions.

Response (7b): Mediterranean.

Under climate change scenarios RCP 2.6 and RCP 4.5, only the Mediterranean biogeographical region is deemed suitable for the establishment of *P. cafer*. The proportion of the region predicted to be suitable increases under both RCP scenarios, to 4% under RCP 2.6 and 6% under RCP 4.5.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): Spain, Belgium, The Netherlands

In the province of Málaga (Spain), breeding was observed in 2001 and 2002, however, it seems that the red-vented bulbul is not established there anymore. The last documented sighting of red-vented bulbul in Malaga province dates back to 2007. In Torremolinos (Málaga), a group of birds was observed in 1998, 2001 and 2002 (A. Paterson, pers. comm.). On November 9, 2000, three individuals were observed singing and on May 27, 2001, a couple copulating. On 7 July 2002, four specimens were observed, of which two were juveniles. On September 9, 2002, a second clutch was confirmed, and the pair was observed with a chick, indicating the species successfully established in this area. However, there are no indications red-vented bulbul is currently still established around Torremolinos (https://www.miteco.gob.es/es/biodiversidad/temas/inventarios-nacionales/).

In Belgium, the red-vented bulbul was observed in August 2005 in the province of Antwerp (http://waarnemingen.be/waarneming/view/41885488). This is the only known observation for Belgium.

In the Netherlands, the red-vented bulbul was observed on five occasions, in September and October of 2006, and in July 2009 (https://data.biodiversitydata.nl/obsint/observation/OBS.44081179).

Response (8b): Spain

In Valencia (Valencia, Spain), the species seems to be established, as breeding was observed in 2017 when a family group of bulbuls were seen in urban gardens surrounding the city (personal communication C. Gutiérrez-Expósito, 12/12/2018). Their range is small, but they are not uncommon. The species is sympatric with a well-established red-whiskered bulbul (*P. jocosus*) population. Successful breeding of red-vented bulbul was reported in 2002 around Torremolinos but there, the last sighting was performed in 2007 (see above).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Cyprus, Spain, Greece, France and potentially other Mediterranean member states with islands (Italy, Portugal, Malta) for which the confidence on the SDM is lower. Under current climate, these countries have suitable areas for the establishment of the red-vented bulbul. Yet the suitable area represents only 2% of the Mediterranean bioregion (see 7a, Annex VII, figure 9).

Overall, the main limiting factor in most of the risk assessment area is annual mean temperature (Bio1). Since *P. cafer* is a species of subtropical climate, it is reasonable to assume that most of Europe will be too cold for successful reproduction. Second, precipitation of the wettest month (Bio13) is the main limiting factor in some parts of southern Europe. The red-vented bulbul indeed avoids deserts and needs trees/shrubs for nesting (Zia et al., 2014), and a low precipitation of the wettest month indicates this. The ensemble model suggested that suitability for red-vented bulbul was most strongly determined by Annual mean temperature (Bio1), accounting for 43.5% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (24.7%), Precipitation of the wettest month (Bio13) (8.8%), Minimum temperature of the coldest month (Bio6) (8.3%), Annual precipitation (Bio12) (5.9%), Human influence index (HII) (5.6%), Precipitation of the driest month (Bio14) (2.3%) and Global tree cover (Tree) (0.9%). For more details, see Annex VII.

Response (9b):

RCP 2.6: Cyprus, France, Greece, Portugal, Spain and potentially other Mediterranean member states with islands (Italy, Portugal, Malta) for which the confidence on the SDM is lower.

RCP 4.5: Cyprus, France, Greece, Portugal, Spain and potentially other Mediterranean member states with islands (Italy, Portugal, Malta) for which the confidence on the SDM is lower.

For more details, see Annex VII.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:

Yes. The red-vented bulbul impacts native biodiversity outside of the risk assessment area in three ways:

1/ Frugivory: most damage relates to its diverse diet that comprises fruits and berries (Islam & Williams, 2000; Brooks, 2013), flowers, buds, insects and small reptiles (Vander Velde, 2002). It feeds on cultivated (food and ornamental) plants and is considered a problematic seed disperser of invasive alien plants, such as *Lantana camara* (Spotswood et al, 2013).

2/ Competition: its aggressive behaviour towards other birds has been reported to lead to niche contraction of some native birds (e.g. Tahiti flycatcher (*Pomarea nigra*) in the Pacific (Blanvillain et al., 2003).

3/ Hybridisation: in the Middle East, crossbreeding with native bulbul species (*P. leucogenys, P. leucotis, P. xanthopygos*) threatens the genetic integrity of these native bird populations (Khan, 1993; Nation et al., 1997; Gregory, 2005; Azin et al., 2008; Khamis, 2010).

On the semi-arid island of Fuerteventura (Canary Islands, Macaronesia, Spain but outside the risk assessment area) the species was first observed in Corralejo in 2003 and expanded its range in the period 2013–2018 to cover the entire island (1.658 km²) (SEO/Birdlife 2017; Nowakowski and Dulisz 2019). There is not a lot of habitat available to the birds on the island, and they are limited to cities and holiday resorts with gardens and parks, but also inhabit agricultural plantations. The first breeding was confirmed in 2018 around Costa Calma. The birds were observed in the treestands (gardens) of a holiday resort composed of various palm trees, fig trees, oleanders, yuccas, acacias and shrubs, and often visited the dry shrubs, typical for semi-arid vegetation of the open landscape of the island, located outside the resort’s gardens (Nowakowski & Dulisz 2019).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:

The red-vented bulbul is currently only present in the Mediterranean biogeographic region (Spain) within the risk assessment area (see Qu. A6b), but has not shown signs of invasiveness (see Qu. A8b).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response:

The red-vented bulbul has only recently established locally in Spain and has not shown signs of invasiveness (see Qu. A.8b).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response:

In its native Indian range, the red-vented bulbul has been reported to feed on the cotton bollworm (*Helicoverpa armigera*), a moth that is globally considered as a major pest species because its larvae feed on a wide range of plants, including many important cultivated crops such as cotton (Rana et al. 2014, 2017).

In a cost-benefit analysis done by Daigneault & Brown (2013) on Fiji, around 47% of surveyed village focus groups reported benefits of the red-vented bulbul for their community. 18% responded that it is effective at insect control, 12% noted that the red-vented bulbul sends out alarms calls when a mongoose is about to attack chickens, thereby reducing the attacks on chickens and another 12% stated that the bulbul is occasionally eaten by villagers. In addition, in the north-east of India, red-vented bulbul fights were part of a traditional and religious annual celebration, until this practice was banned in January 2016 (Shalet, 2016).

As the species is widely kept as a caged bird within and outside the risk assessment area (see A.2), it represents ornamental, sentimental and aesthetic value as a companion animal. There are no official records on trade volumes, but there are plenty of advertisements for birds online, often sold in pairs. Prices found online vary from € 165 – 250 per bird or € 250 per pair.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: "No information has been found."
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as "corridor" or "unaided)".
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[3] and the provided key to pathways[4].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions

1.2-1.9

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[3] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[4] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Pathway name:

a. ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)
b. ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)
c. TRANSPORT stowaway: Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Qu. 1.2a. ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)

Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**intentional** unintentional	**CONFIDENCE**	low medium **high**

Response:

Intentional because zoological gardens will intentionally buy or acquire one or more red-vented bulbul individuals to put on display.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Response:

Whilst there is no data available on the total captive red-vented bulbul population within all zoological collections within the EU, information was provided by EAZA (European Association of Zoos and Aquaria) on populations kept at approximately 300 of their Member zoos and aquariums in 26 EU Member States (with the exception of Cyprus and Malta). The information provided by EAZA (EAZA datafile 3/10/2019) indicates that the species is kept in low numbers by EAZA Member zoos in Germany, Poland and the UK. This data comes from the animal care and management software provided by Species360 Zoological Information Management System (ZIMS) (zims.Species360.org, 2018) whose usage is widespread throughout the EAZA Membership. It must be noted that actual situation might slightly differ if the species has been recorded under a different/older taxonomic name.

The red-vented bulbul is on display in at least the following EAZA zoos in the risk assessment area: Warsaw Zoological Garden (Poland), Köln Zoologischer Garten (Germany), Plzen Zoo (Czech Republic), Graested Nordsjællands Fuglepark (Denmark), Helsingborg Djurparken / ex. Fågelparken (Sweden), Farnham Birdworld & Underwater World (UK), Thrigby Hall Wildlife Gardens (UK) (www.zootierliste.de). EAZA is the European Association of Zoos and Aquaria and has 300 full members, 21 candidates for membership, 40 corporate members and 38 associate members as of May 2018 (www.eaza.net). This list comprises just a quarter of all zoological gardens and animal parks in Europe (www.zoos.media). Therefore, it is difficult to assess to what extent the species is kept in captivity within the risk assessment area.

Although the import of wild birds into the EU has been illegal since 2005, zoological gardens with a special zoo license can still import them. Indeed, the red-vented bulbul is present in several zoos, but it is only moderately likely that large numbers will be introduced in the zoos within the risk assessment area over the course of one year. However, no information is available as to the total number of zoos that keep red-vented bulbul, the size of the captive population nor how often these are introduced into a new zoo, so the confidence level is low.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

If a zoo intentionally buys or acquires one or more red-vented bulbul individuals, it is very likely that these animals will survive their transport and storage along this pathway, since they are meant to stay alive. Reproduction however, is very unlikely.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	very unlikely unlikely	**CONFIDENCE**	low medium

	moderately likely likely **very likely**		**high**

Response:

When imported legally by a zoological garden with a license, there is no reason why existing management practices would target this species. In the case of illegal import, there are no known existing management practices that target the red-vented bulbul.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**very unlikely** unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

Response:

The receiving zoo will always know that they are introducing the red-vented bulbul. Additionally, when on display, visitors will also detect this bird.

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

The red-vented bulbul is already introduced in the risk assessment area, as it is present in several zoological gardens. Since zoological gardens can acquire a license to import wild birds and this species is not considered threatened, we can say with high confidence that it is very likely that the red-vented bulbul will be introduced into the risk assessment area through this pathway.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.2b. ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for

such species)

Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	**intentional** unintentional	**CONFIDENCE**	low medium **high**

Response:

Intentional because a person will intentionally buy the red-vented bulbul to keep as a pet even if the subsequent escape would be accidental (following IUCN 2017).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Response:

On several hobbyist websites, it is stated that the red-vented bulbul is among the most easily-kept of the softbills, which is a term applied to non-typical cage birds, such as bulbuls. The species has a confident and inquisitive nature, with a “friendly” personality towards humans, making them popular as pets. As stated in Qu. A13, there are no official records on trade volumes, but there are plenty of advertisements for birds online, often sold in pairs. Prices found online vary from € 165–250 per bird or € 250 per pair. Wild caught bird trade (as opposed to captive bred) has been suspended in the EU since 2005, when a temporary ban on wild bird imports was installed to prevent the spread of avian influenza (Reino et al., 2017;). The ban was made permanent in 2007 and considers all wild caught bird imports regardless of species’ conservation status. This ban has been effective in reducing propagule pressure (Cardador et al. 2019). We did not find any records of illegal trade into the EU from non-EU countries, however the red-vented bulbul is traded between hobbyists within Europe. We did not find information on the number of the red-vented bulbul present in the risk assessment area, which makes it impossible to assess propagule pressure. Neither do we know how many birds are exchanged/traded between hobbyists, so we also have no idea of the market for animals bred in captivity. Our confidence is therefore low.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

Response:

As stated in Qu. 1.3b, we do not know if the red-vented bulbul is imported into the EU illegally, making it very difficult to assess if many birds die during transport. We can assume though that the intention is to bring live animals with the aim to keep them and make them reproduce in captivity. Likewise, in the case of exchange/trade between hobbyists, their intention is to keep the birds alive and well during transport to deliver them so survival is likely. Since we have no official records on this matter, confidence is low.

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low **medium** high

Response:

In the case of trade between hobbyists, this is a legal trade pathway with the intention to keep the birds alive and well during transport to deliver them to the buyer. As stated above, we do not know if the red-vented bulbul is imported into the EU illegally. We also do not know of any existing management practices that could possibly kill the red-vented bulbul during transport and storage along this pathway. According to the IUCN, the red-vented bulbul is not subjected to any international management or trade controls (IUCN, 2019). Because of this, we think it is very likely that the red-vented bulbul will survive existing management practices, with medium confidence.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Response:

The red-vented bulbul has been introduced without being detected at first in several parts of its invasive range in the Pacific and the Middle East (Watling, 1978; Vander Velde, 2002). However, we could not find evidence on any undetected introductions of the red-vented bulbul into the risk assessment area, so confidence is only medium.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Response:

This species is already present as a pet in private collections in several (if not all) countries within the risk assessment area. Several hobbyist websites currently have the species on offer, suggesting it is very likely to be introduced over and over again in the future within the risk assessment area. However, information on introduction from outside the risk assessment area is lacking, therefore our response is moderately likely with low confidence.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.2c. TRANSPORT stowaway: Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional **unintentional**	**CONFIDENCE**	low medium **high**

Response:

If the red-vented bulbul were to hitchhike on cargo ships or fishing boats, then this would be unintentional transport.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Response:

Some of the introductions of red-vented bulbul on the Marshall Islands could possibly be stowaways on container ships or fishing boats (Vander Velde, 2002; Brochier et al., 2010). Locations of first sightings in Majuro were all in close proximity to its major commercial port. Here, containers enter from Hawaii and Asia, where there are resident populations of red-vented bulbul. There are also speculations that introductions in Hawaii and New Zealand (Auckland in the 1950s) could have been assisted by barge or boat (Islam & Williams, 2000; Heather & Robertson, 1996).

Red-vented bulbuls are known to nest in some unusual places, including the motor of a ceiling fan and the end of a curtain rod, both within buildings (Islam & Williams, 2000). Hence, the possibility exists that a few birds stowed away among some heavy equipment or in crevices on board of a ship (Vander Velde, 2002). However, there are no confirmed stowaways so the confidence level of our answer is low.

This type of introductory event is considered to be moderately likely, since it possibly already happened outside of the risk assessment area. However, there are no official records of this, so confidence level is low.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

Response:

see Qu. 1.3c.

In the past, some birds were stowaways and survived on a ship from Hawaii to Majuro (Marshall Islands), a journey of around 3700 km (Vander Velde et al., 2002). Birds from the population on the Arabian Peninsula could possibly hitchhike to Europe through this pathway (the distance from Dubai to Cyprus is around 6000 km). There is also a population on Fuerteventura, one of the Canary Islands (Spain) (Nowakowski and Dulisz 2019). The journey from Fuerteventura to Cádiz (Spain) is just over 1100 km, which probably is perfectly manageable for the red-vented bulbul.

When it comes to food supply for survival, if a couple of red-vented bulbuls have been able to survive a journey of 3700 km (Vander Velde, 2002), they probably found food or were fed along the way, indicating this could also be possible for journeys of 6000 km or more.

Qu. 1.5c. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

Response:

There are no known management practices for these birds on ships, hence it is likely to survive transport and storage along the pathway.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	**low** medium high

Response:

It is unlikely stowaway red-vented bulbul on cargo ships or fishing boats would remain undetected during the journey. These birds are noisy, active and curious (Vander Velde, 2002). Nonetheless, even if the birds would be detected, there is no certainty that this sighting would ever be reported by the sailors. Since there are no official records of this happening, the confidence is low.

Qu. 1.7c. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Response:

This has possibly happened in the past in the Pacific Ocean, not in the risk assessment area. There are no official records so the confidence is low.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	very unlikely unlikely	**CONFIDENCE**	low medium

	moderately likely likely **very likely**		**high**

Response:

This species is already present in zoological gardens and private collections in multiple countries within the risk assessment area. We did not find any quantitative data though. Since there is a ban on the introduction of wild birds into the EU, from which only zoological gardens with a license are exempted, it is most likely that the red-vented bulbul will be introduced through the first pathway. Introductions into private collections are less likely, and introductions as stowaways will be the least likely. There are no indications of differences between biogeographical regions.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

The likelihood of introduction will not change in any climate change scenario since none of the pathways will be affected by any of these scenarios.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[5] and the provided key to pathways[6].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name:

a. RELEASE in nature: Landscape/flora/fauna “improvement” in the wild
b. ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)
c. ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)
d. TRANSPORT stowaway: Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Qu. 2.2a. RELEASE in nature: Landscape/flora/fauna “improvement” in the wild

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	**intentional** unintentional	**CONFIDENCE**	low medium **high**

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[5] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[6] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Response:

If the red-vented bulbul were to be released from its confinement, then that would be intentional, as it is a release and not an escape. In several Asian countries (China, Vietnam, Malaysia, Thailand, Korea, Cambodia, possibly more), people "make merit" by releasing captive animals (McNeely, 2001). "Making merit" is a Buddhist practice that determines the quality of the next life and contributes to a person's growth towards enlightenment. However, IAS are also released, simply due to the fact that the people involved do not know about IAS (McNeely, 2001; Severinghaus & Chi, 1999).

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Response:

This has happened in the past outside the risk assessment area, for instance in the Pacific islands, where Indian immigrants released red-vented bulbuls to avoid persecution after illegally importing them for bird fights (Parham, 1955; Watling, 1978; Gill et al., 1995). At the moment, there are no records from illegally imported red-vented bulbuls in the risk assessment area. It is however possible that release will happen again, for instance by activists or the pet owner wanting to free the bird, not being able to take care of it or after overly successful breeding.

Confidence level is medium since this has been recorded in the past but in the risk assessment area there are no records.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**very unlikely** unlikely moderately likely likely very likely	**CONFIDENCE**	low **medium** high

Response:

Apart from the person releasing the bird(s), it is likely that other people will also detect the red-vented bulbul if present in the wild. This is a loud, active and gregarious bird which is not readily confused with any native European species. Besides, the red-vented bulbul prefers anthropogenic habitats like urban gardens and parks, adding to its detection rate. In addition, given the popularity of bird watching, early sightings are quite likely to arise through bird watching reports.

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	**low** medium high

Response:

It is very likely that the person releasing the red-vented bulbul will do this when the weather is appropriate, given that this person knows the environmental requirements of this bird. However, since this has not been recorded, the confidence of our statement is low.

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low **medium** high

Response:

If the release event takes place in one of the countries listed above (Qu. A9) where climate is suitable for the red-vented bulbul, then transfer to suitable habitat will likely not be a problem. Two factors are important in this case, the first one being that the person releasing the bird wants it to survive, and the second one that this species is known to thrive in anthropogenic landscapes, like urban parks and gardens (Vander Velde, 2002), which are widespread in the risk assessment area. In addition, these parks and gardens are more likely to offer introduced fruity plants from its native area on which the red-vented bulbul can feed. Supplementary feeding stations in gardens could also increase the birds chances of survival and reproduction.

There are no records of release events within the risk assessment area, so confidence level is low.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Response:

Based on what has happened in the past outside the risk assessment area (in several Pacific islands for example) (Thibault et al., 2018a), it can be assumed that there is a possibility of the red-vented bulbul being released within the risk assessment area as well. However, no records from within the risk assessment area are present so confidence is medium.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.2b. ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from confinement)?

RESPONSE	intentional **unintentional**	**CONFIDENCE**	low medium **high**

Response:

Escape, so entry into the environment is unintentional with high confidence.

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Response:

The red-vented bulbul is present in several EAZA zoological gardens throughout Europe (see Qu. 1.3a). These are all located in Northern or Central Europe, not in Southern Europe, where we expect more suitable habitat and climate for red-vented bulbul. However, as stated above, EAZA only represents a quarter of all European zoological gardens, so there might be some in zoological gardens in Southern Europe.

There is a record of a zoo escape on Fuerteventura (Canary Islands), outside of the risk assessment area. Here, birds escaped from the zoological garden of La Lajita in 2013 (together with common myna *Acridotheres tristis*), settled and started to breed in the surroundings a few years later (Nowakowski and Dulisz 2019). The myna was eradicated from the surroundings of the zoo, yet the bulbuls were left unattended and spread to the rest of the island (Nowakowski and Dulisz 2019). Considering the potential impact on biodiversity of the Canary Islands ecosystems, including by predation on native and endemic species, there are calls for its eradication (SEO/Birdlife 2017).

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**very unlikely** unlikely moderately likely likely	**CONFIDENCE**	low medium **high**

	very likely		

Response:

It is very unlikely that the red-vented bulbul would be able to escape from a zoological garden without this being noticed. In the event of an escape, the zoo will most likely take measures to recapture the animal. However, a study by Cassey & Hogg (2014) in Australia stated that, compared with mammals and reptiles, bird escapes were significantly less likely to be retrieved, and more likely to not be retrieved.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	**low** medium high

Response:

Mean annual temperature is the limiting factor for most of the risk assessment area. If the red-vented bulbul would escape in northern Europe, then it would likely enter a habitat which is too cold in autumn, winter and spring. The bird could possibly survive if it would enter into the environment during summer.

For more information on climate suitability see Qu. A7.

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Response:

Zoological gardens are often located in urbanized areas, meaning that if the red-vented bulbul would escape, it would easily find an urban garden or park in which to establish. As mentioned in Qu. 2.5b, if the zoological garden is located too northerly in Europe, the climate will be too cold during autumn, winter and spring.

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Response:

If the red-vented bulbul is present in zoological gardens within its predicted suitable habitat, then it is moderately likely that the species would enter into the environment within the risk assessment area. Given that we do not have information on the presence of the red-vented bulbul in zoological gardens within these suitable areas, the confidence level is low.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.2c. ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from confinement)?

RESPONSE	intentional **unintentional**	**CONFIDENCE**	low medium **high**

Response:

Escape, so entry into the environment is unintentional, with a high confidence level.

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after

	eradication
●	if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low **medium** high

Response:

The red-vented bulbul is probably kept as a pet in most (if not all) countries within the risk assessment area (see Qu. 1.7b) and the species can easily be found on display for sale online, often under the name "Kala buulbuul" (e.g. http://buulbuul.nl/). It is known as a hardy species to keep in aviaries, which is easy to breed on a variety of mealworms and insects when they have young. Several hobby-keeper websites indicate that red-vented bulbul wings should not be clipped, since they exercise by flying, not by climbing. Some websites indicate the species should be kept in a large walk-in aviary, preferably outdoors, implying a higher chance of escape compared to birds kept inside in small cages. Additionally, red-vented bulbuls are often sold and kept in pairs, which implies that there is a high chance of breeding when they would escape. Shieh et al. (2006) reported that Pycnonotidae (with Sturnidae, Timaliidae and Cacatuidae) have significantly higher probabilities of escaping from captivity in Asia, in comparison to other birds families.

This sort of escapes has been recorded in the past in the Netherlands and Belgium (see Qu. 2.3a and Qu. A6).

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	low medium **high**

Response:

If the red-vented bulbul would escape, it is likely that the keeper will alarm neighbours and maybe even animal rescue centres nearby. For more info see Qu. 2.4a.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Response:

The red-vented bulbul is a species of equatorial climate, and will most likely only be able to establish in the Mediterranean biogeographical region within the risk assessment area. For more information on climate suitability see Qu. A7 and the SDM.

If this species would escape from its confinement in the south of Europe, then any moment of the year will probably be appropriate for establishment. The species is known to settle in urban areas, where temperatures are higher and food is more readily available, increasing its chances of establishment (Vander Velde, 2002).

In fact, there have been two successful entry events for the red-vented bulbul in the risk assessment area: in Málaga and in Valencia. In Valencia, there has been establishment following the entry of the species. It is possible that these birds escaped, but this has not been recorded.

Confidence level is medium because of the lack of official reports on the matter within the risk assessment area.

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Response:

See Qu. 2.6b.

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low medium **high**

Response:

This sort of event has happened (and was recorded) multiple times in the past outside the risk assessment area. Moreover, this has happened inside the risk assessment area too, but records of this are scarce and not official. The confidence level of our response is still high because of the records outside of the risk assessment area.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.2d. TRANSPORT stowaway: Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from confinement)?

RESPONSE	intentional **unintentional**	**CONFIDENCE**	low medium **high**

Response:

Birds that hitchhike on a ship/boat would enter unintentionally.

Qu. 2.3d. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	**low** medium high

Response:

This sort of event has allegedly happened before in the Pacific (Vander Velde, 2002). However, the number of birds that would actually be able to enter the environment through this pathway over the course of one year will be very low, therefore we score unlikely. Since we have no official records of this happening, our confidence is low.

For more information, see Qu. 1.3c.

Qu. 2.4d. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**very unlikely** unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

Response:

See response Qu. 1.6c.

Qu. 2.5d. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Response:

The red-vented bulbul is a species of equatorial climate, and will most likely only be able to establish in the Mediterranean biogeographical region within the risk assessment area. For more information on climate suitability see Qu. A7 and the SDM (Annex VII).

There are populations of the red-vented bulbul on Fuerteventura (Canary Islands) and on the Arabian Peninsula, which are in fact closest to the areas within the risk assessment area where it is most likely that the red-vented bulbul could establish. For additional information regarding months of the year most appropriate for establishment, see response Qu. 2.5c.

Qu. 2.6d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	low **medium** high

Response:

See response to Qu. 2.6b.

Qu. 2.7d. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely **moderately likely** likely very likely	**CONFIDENCE**	**low** medium high

Response:

This sort of event has probably happened in the past (not in the RA area), but has not been recorded very well, hence the low confidence.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions. Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low **medium** high

Response:

Entry has happened in several locations within the risk assessment area (at least in the Netherlands, Belgium, Spain, see Qu. A8). Since the red-vented bulbul is kept as a pet and is on display in zoological gardens, escape and release events are bound to occur in the future. Additionally, it is possible that some individuals or pairs will hitchhike with boats coming from areas with a red-vented bulbul population. Entry could happen in all biogeographical regions within the risk assessment area, but establishment will only be possible in the Mediterranean region. For additional information, see Qu. A7 and the SDM.

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low **medium** high

Response:

Climate change will not alter the possibility of entry into the environment, so we score this question the same as we scored the question under current climate. For additional information, see Qu. A7 and SDM (Annex VII).

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

The red-vented bulbul is a species of an equatorial climate but is also established in cooler and drier climate regions (including the Mediterranean biogeographical region), both in and outside of the risk assessment area. It has so far primarily established in urban areas, of which there is no shortage within the risk assessment area.

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	very isolated isolated **moderately widespread** widespread ubiquitous	**CONFIDENCE**	low **medium** high

Response:

The climatic niche of the red-vented bulbul corresponds to an equatorial climate according to the Koppen-Geiger classification (Kottek et al. 2006). In the Mediterranean biogeographical region, suitable habitat for the red-vented bulbul is moderately widespread. Under current climate the area predicted suitable represents only 2% of the entire Mediterranean bioregion but is likely to increase under future climatic conditions (see 7a,b; Annex VII, figure 9). Especially the southern islands of

Greece, the area around Gibraltar and the Atlantic coast of Portugal are at risk. With a non-native range that is still expanding, there is a possibility that the potential climatic niche hence the predicted potential distribution is underestimated.

The red-vented bulbul feeds on a variety of fruits, berries, flowers, buds, insects and small vertebrate prey. This broad diet, that includes cultivated plants allows the red-vented bulbul to find food easily. Red-vented bulbul builds its nest in trees and bushes at different heights, either on the forks of trees, in the middle or at the top (Vijayan, 1980; Zia et al., 2014). According to several studies, preferred nest height varies from 1 - 4 meters and preferred trees are thorny and very close to each other (Vijayan, 1980; Watling, 1983; Zia et al., 2014). The study done by Zia et al. (2014) in the native range in India found that the percentage of failed nests was highest for treetop nests, with most nests being destroyed due to heavy wind, rain or predators. Such thorny trees are omnipresent in the Mediterranean, not the least on the Greek islands, therefore red-vented bulbul has plenty of suitable breeding habitat available in the Mediterranean biogeographical region.

It is unlikely that the red-vented bulbul would be able to establish in the northern Atlantic, Boreal, or Continental biogeographical regions, due to the cold winter (Annex VII). This is corroborated by reports from a keeper in The Netherlands who says that several young died when kept outside due to the cold weather during the night (http://www.buulbuul.nl/Mijn%20Kala%20buulbuul.html).

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	**N/A** very unlikely unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

Response:

The red-vented bulbul does not require another species for any critical stage of its life cycle.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low **medium** high

Response:

Potentially, the red-vented bulbul could compete for food and space (e.g. for nesting) with other bird species. However, as establishment often occurs in (sub)urban areas with gardens, parks and artificial habitat (see A8, A10), usually depleted in local fauna, very low levels of competition with native species can be expected and it is very likely establishment will occur despite this. In the risk assessment area, competition with highly successful native species such as corvids, gulls and starlings could impact establishment in some cases, but there have been no studies that have assessed this so far hence medium confidence.

It should be noted that the red-whiskered bulbul (*P. jocosus*) established around Valencia shortly after the first observation was made in 2003 in the lower Rio Turia basin area (Santos 2015). The red-whiskered bulbul has been reproducing for more than a decade here and was estimated at 100-150 individuals in 2016 (Santos 2015). Detailed impact studies for Spain are equally lacking for this species. However, as the two species of bulbul are now sympatric, there is the possibility of cumulated impact of an entire invasive bird community. Introduced species may act in concert, facilitating one another's invasion, and increasing the likelihood of successful establishment, spread and impact. Such positive interactions among introduced species are relatively common (e.g. between birds/mammals and plants), but few have been studied in detail (Traveset & Richardson 2014). No information on such mechanisms is available for bulbuls.

The red-vented bulbul is known to be aggressive towards other birds in its preferred forage trees, especially during the breeding season (Sherman & Fall, 2010; Blanvillain et al., 2003; Gorman 1972). Competition with other (native) bird species is in fact one of the three serious impact categories associated with red-vented bulbul, so it is unlikely that competition will be limiting for this bird (Thibault et al., 2018d). For example, in Tahiti, red-vented bulbuls compete with the Tahiti monarch (*Pomarea nigra*), an endemic and critically endangered passerine (Blanvillain et al., 2003). Another study, done by Thibault et al. (2018d) in New-Caledonia found that nine out of ten native bird species monitored in man-modified habitats were less abundant when the red-vented bulbul was present. The impact of the red-vented bulbul appears to be restricted to niche contraction of the native species (Thibault et al., 2018d).

A study in New Caledonia (Thibault et al., 2018d) states that there are no indications for interspecific competition with other invasive species present on the island, such as common myna (*Acridotheres tristis*) and red-whiskered bulbul. It is possible that these species show some sort of niche segregation in their invaded range, as was shown for red-vented bulbul and common myna in French Polynesia by Bates et al. (2014).

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely	**CONFIDENCE**	low medium **high**

	moderately likely **likely** very likely		

Response:

Predators

The red-vented bulbul is not particularly vulnerable to predation. In its native range, a study carried out by Zia et al. (2017) found that predation rate on red-vented bulbul was only 6% in eggs and 9% in nestlings. Predators in the study area included raptor species like crested eagle (*Morphnus guianensis*) and barn owl (*Tyto alba*), and rodents like the black rat (*Rattus rattus*). Breeding success in the study was 82% and 86% for eggs and fledglings, respectively. The red-vented bulbul has specific behavioural adaptations to avoid predatory impact during nesting, notably through broken-wing display, that one or both parents would perform when a predator is seen near the nest (Kumar 2004).

Parasites

In its native range, the red-vented bulbul is known to host the internal parasite *Isospora* spp. known to cause isosporiasis in passerine birds (Boughton et al. 1938), lice species like *Menacanthus eurysternus* (Price 1975), *Bruelia guldum*, *Sturnidoecus guldum* (Ansari 1957), the mite *Pteroherpus pycnonoti* (Constantinescu et al., unpublished) as well as disease carrying ticks (Islam & Williams, 2000; Vander Velde & Vander Velde 2013; Thibault et al. 2018c). Vander Velde and Vander Velde (2013) considered the constant influx of red-vented bulbuls on Micronesia a potential risk for the spread of tick-borne diseases.

Pathogens

In 1996, Jarvi et al. (2003) detected no avian malaria (*Plasmodium spp.*) in blood smears, and Atkinson et al. (2006) found no evidence of *Plasmodium, Trypanosoma, Atoxoplasma* or microfilaria. Red-vented bulbuls in Tahiti, however, have been found to carry the zoonotic disease *Chlamydia sp.* (Blanvillain et al. 2013). Grewal (1964) experimentally infected red-vented bulbuls with *Plasmodium praecox* (= *relictum*), the most widespread malaria parasite of birds. The birds developed typical infections about a week after inoculation and survived without apparent. The species may therefore be a carrier in nature (Grewal 1964).

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

There are no known existing management practices against this species in the risk assessment area.

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	**very unlikely** unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

Response:

There are no known existing management practices against this species in the risk assessment area.

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely **unlikely** moderately likely likely very likely	**CONFIDENCE**	low **medium** high

Response:

As stated before, the red-vented bulbul is a loud species and it is thus unlikely to stay undetected. It is in fact this biological property that will make it more susceptible to eradication campaigns.

The most suitable habitat for the red-vented bulbul are the Greek islands. Invasive alien species control tends to be more achievable on islands than on the continent (Myers et al. 2000; McGeoch et al. 2016).

For more information, see the Annex with control measures.

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

The red-vented bulbul lives in anthropogenic landscapes in all of its alien range in the Pacific, Houston, Fuerteventura, Málaga and Valencia, so it can be assumed that the highly fragmented and anthropogenic landscape in southern Europe will not hamper its establishment.

If a pair escapes or is released from confinement, a population could establish from just this pair given that genetic diversity is sufficiently high. This is allegedly what happened in the Republic of the Marshall Islands, when a pair may have hitchhiked on a ship and established a population (Vander Velde, 2002).

The red-vented bulbul often has two to three broods per year, that consist of two to five eggs (Long, 1981; Vander Velde, 2002), with an incubation period of about 14 days (Berger, 1972). Consequently, population size is likely to increase fast, leading to establishment.

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

The red-vented bulbul is a species that thrives in urban and suburban gardens and parks, indicating that it is an adaptable species (Brooks 2013, Thibault 2018a). Thibault (2018a) showed that densities in suburban areas vary along an urbanization gradient, but can go up to 120 individuals/km^2. In their native range, bulbuls are found from 0 to 2,000m, along forest edges, as well as in gardens and cultivated areas. These habitats have plenty of exotic plant species available to red-vented bulbul, usually not or little consumed by local wildlife, a resource that can easily be exploited by these adaptable birds and offering some advantage over native passerines. Virtually all of the bulbuls in Houston are found in residential gardens at sea level, with the only other cases being fragments of secondary habitat in edge situations.

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low medium **high**

Response:

No genetic diversity studies have been done on the red-vented bulbul, but several studies on singular breeding species (i.e., species that breed in pairs on a defended territory) show that these do not avoid random mating (Van Tienderen & van Noordwijk, 1988; Keller & Arcese, 1998; Hansson et al., 2007). However, Kruuk et al. (2002)) noticed severe inbreeding depression in collared flycatchers, indicating species-specific differences in inbreeding tolerance.

Even with a small founder population, the red-vented bulbul has established in several parts of the world where it is thriving (also see Qu. A5).

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

Since the red-vented bulbul is a popular pet bird and is present in zoological gardens, there is a continuous risk of release and escape in the future. Actual recurring introduction, entry and release events without establishment will happen in areas with unsuitable climatic conditions, e.g. in many colder parts of Europe, where it is too cold for the red-vented bulbul to reproduce successfully.

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low **medium** high

Response:

As stated in Qu. A7 and the SDM (Annex VII), establishment is most likely in the Mediterranean biogeographical region but only a small proportion of the area is predicted suitable. Indeed, establishment has already happened in Valencia (Spain). This area has similar climatic conditions as the current distribution area (arid and mediterranean). The ensemble model (Annex VII) suggested that the suitable distribution area for red-vented bulbul was most strongly determined by Annual mean temperature (Bio1), accounting for 43.5% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (24.7%). Annual mean temperature (Bio1) was also the most strongly limiting factors for establishment of red-vented bulbul in most of Europe and the Mediterranean region in current climatic conditions. In some Mediterranean areas (southern Iberia, Balearic islands, Sicily and Sardinia, Greece and Aegean islands, Cyprus), Precipitation of the wettest month (Bio13) was the most limiting factor. Other climatic variables such as Precipitation of the wettest month (Bio13) (8.8%), Minimum temperature of the coldest month (Bio6) (8.3%), Annual precipitation (Bio12) (5.9%) and Precipitation of the driest month (Bio14) (2.3%) explained much less of the variation in the species distribution model. The considered Non-climatic factors Human influence index (HII) (5.6%) and Global tree cover (Tree) (0.9%) explained only little of the observed variation. For information about important non-climatic variables, see Qu. 3.14.

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable

climate change conditions: explaining how foreseeable climate change conditions will influence this risk.
With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	low **medium** high

Response:

As stated in Qu. A7 and the SDM (Annex VII), establishment is estimated to be possible in the Mediterranean biogeographical regions under both RCP 2.6 and RCP 4.5. The Mediterranean will remain the most vulnerable region under climate change.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic “spread” via release or escape (“jump-dispersal”), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	low **medium** high

Response:

For information on spread outside of the risk assessment area, see Qu. A5. For information on spread inside of the risk assessment area, see Qu. A8(b). There is a discrepancy between spread rate inside and outside of the risk assessment area, the rate being higher outside than inside. The rate of spread will be dependent on propagule pressure and the size of the population that has established.

Life history traits important for spread: the breeding season in the red-vented bulbul starts in February and lasts till September (Zia et al., 2014). Nest construction period is only 2 - 5 days, which is notably faster than other Pycnonotids like yellow-throated bulbul and grey-headed bulbul for which nest building takes 3 - 8 days (Balakrishnan, 2010). According to a study performed by Zia et al. (2014), preferred nest-building vegetation of the red-vented bulbul was beri (*Zizyphus nummularia*) (31%) followed by guava (*Psidium guajava*) (22%), sheesham (*Dalbergia sissoo*) (18%), snatha (*Dodonea viscosa*) (16%) and date palm (*Phoenix dactylifera*) (13%). Clutch size in general in Pycnonotids is two and rarely three (Ali & Ripley, 1987). Clutch size of the red-vented bulbul varies from 1 - 4, and there are indications that this varies between regions, as studies from different regions of the species' range have partially different results (Zia et al., 2017; Prajapati et al., 2011; Rao et al., 2013).

The study done by Zia et al. (2014) also recorded the predators of the red-vented bulbul nests. Mostly, rodents and raptors were responsible for failed nests, e.g. brown rat (*Rattus rattus*), barn owl (*Tyto alba*) and crested eagle (*Morphnus guianensis*). Another interesting found in the study was that nests made in beri plants were more likely to fail which could be connected to their location near residential areas, where local pollution could have an effect on red-vented bulbul reproductive success.

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

Response:

The red-vented bulbul is known to nest in unusual sites (see Qu. 1.3b), and it could therefore spread by human assistance on cargo ships or fishing boats within the risk assessment area. It appears that this species preferentially spreads through urban corridors, possibly facilitating its spread in most European countries.

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.

- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name:

TRANSPORT (stowaway) - Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional **unintentional**	**CONFIDENCE**	low medium **high**

Response:

Unintentional.

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

Response:

Introduction, establishment and spread of the red-vented bulbul has allegedly happened through this pathway in the Pacific, suggesting that this could happen in the risk assessment area as well (Vander Velde et al., 2002). The red-vented bulbul was first sighted on the Marshall Islands in 2000 near the major commercial dock of Majuro and in 2002, there were already several breeding populations (Vander Velde, 2002).

The Mediterranean region and the Greek islands in particular are vulnerable given the large amount of islands and boat (commercial and leisure) traffic between them.

Also see response to Qu 1.3c and 1.4c.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

Response:

This sort of event has allegedly already happened in the Pacific (Vander Velde et al., 2002), but evidence is scarce, hence the low level of confidence. See Qu. 1.3c & 1.4c.

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely **very likely**	**CONFIDENCE**	low medium **high**

Response:

There are no known existing management practices on ships that would target the red-vented bulbul.

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**very unlikely** unlikely moderately likely likely very likely	**CONFIDENCE**	low medium **high**

Response:

Also see Qu. 2.4a.

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very unlikely unlikely moderately likely **likely** very likely	**CONFIDENCE**	**low** medium high

Response:

If the organism would spread through the Mediterranean by boat transport, it would enter new areas through harbours, which are often close to urbanisation. This would enhance its chances of spread, since the species is known to settle in urban areas (Vander Velde, 2002), where temperatures are higher and food is more readily available, increasing its chances of establishment.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	very slowly **slowly** moderately rapidly very rapidly	**CONFIDENCE**	**low** medium high

Response:

Spread through this pathway has possibly happened in the Pacific, and could happen in the risk assessment area, especially in the Mediterranean region. However, there are no official records of this so confidence is low.

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in

relation to these pathways of spread?

RESPONSE	very easy **easy** with some difficulty difficult very difficult	**CONFIDENCE**	low **medium** high

Response:

The species is easily recognisable and will probably be detected when present on a ship. We found no information on standard measures that sailors take to prevent the birds from escaping the ship. We did find some recommendations from the Australian government for sailors that encounter a stowaway animal on a ship: closing container or vessel doors, creating barriers; isolating the affected cargo in an area away from other goods; using blankets to restrict animal movement; taking photos of the animal or try to catch it.

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	very slowly slowly **moderately** rapidly very rapidly	**CONFIDENCE**	low **medium** high

Response:

Groups and families of the red-vented bulbul can be seen in Valencia, but their range is small (Carlos Gutiérrez-Expósito, personal communication 2018), suggesting that spread is slow. For additional information, see Qu. A8.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly **moderately** rapidly very rapidly	**CONFIDENCE**	low **medium** high

Response:

The species is classified as a rather sedentary species, showing possible movements depending on environmental conditions (del Hoyo et al. 2005). The invasion on Fuerteventura, where the species invaded the entire island in a few decades and has shown important range expansion in the period 2013–2018 (Nowakowski and Dulisz 2019) indeed shows spread could be very context-dependent and the species is certainly able to spread moderately rapidly or rapidly in insular contexts.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	low medium **high**

Comment:

Impacts on biodiversity were comprehensively reviewed by Thibault et al. (2018a) and include impact on native fauna through competition and community changes due to dispersal of invasive alien plant seeds. The authors note the lack of quantified impact studies and based on this, challenge the inclusion of red-vented bulbul on the list of the world's worst invasive species (Lowe et al. 2004). Evans et al. (2016), in their EICAT assessment of 415 bird species, scored impact of the red-vented bulbul as Moderate (MO) (i.e. *it causes declines in the population size of native species, but no changes to the structure of communities or to the abiotic or biotic composition of ecosystems*) with high confidence and list competition and interaction with other alien species (spreading the seeds of alien plants and thereby mediating alien plant invasions) as the impact mechanisms causing the most severe impacts. Martin-Albarracin et al. (2015) and Baker et al. (2014), in a global analysis of alien bird impact, note competition and interactions with other non-native species as impact mechanisms. According to their scoring system used (an adapted scheme based on Kumschick and Nentwig (2010) and Blackburn et al. (2014), where 0 indicates no impact detected and 5 massive impact), the red-vented bulbul scores 2 on competition (i.e. *competition with several native species by exploitation competition, without large impact on affected species or decline of their populations*) and 4 on interactions with other species (i.e. *dispersal of seeds of non-native plants facilitates local or population extinction of at least one native species, and produces changes in community composition that are reversible but would not have occurred in the absence of the species*).

Impact mechanisms:

1: Frugivory (spreading the fruit/seeds of alien plants)

Thibault et al. (2018c) compiled lists of 110 plant species consumed and 33 plant species dispersed by red-vented bulbul. In the literature there are at least 56 mentions of problematic seed dispersal by the species from eight locations (six countries) (Thibault et al. 2018c). Red-vented bulbul is considered a major vector of some notoriously problematic invasive alien plant species on islands, such as the invasive tree miconia (*Miconia calvescens*) and lantana (*Lantana camara*) in French Polynesia (Meyer, 1996; Spotswood et al., 2012; 2013), lantana (*Lantana camara*), prickly solanum (*Solanum torvum*) and cape gooseberry (*Physalis angulata*) on Fiji (Fox, 2011), ivy gourd (*Coccinia grandis*) on Oahu and brazilian pepper (*Schinus terebinthifolius*) in New Caledonia (Spotswood et al., 2012; Thouzeau-Fonseca, 2013).

2: Competition

Blanvillain et al. (2003) provide evidence of competition with Tahiti monarch (*Pomarea nigra*), a forest bird endemic to Tahiti (French Polynesia). They noted aggressive interactions (e.g. alarm calls and chasing) between flycatchers and red-vented bulbuls during and outside reproductive activity and suggest this interspecific competition for nest sites and territories might be in part responsible for low breeding success of the Tahiti flycatchers. In contrast, interactions with common myna (*Acridotheres tristis*) were more common when the birds had chicks and eggs in the nest and therefore mynas had a more important direct impact as nest predators of the monarchs (Blanvillain et al. 2003). Competition between red-vented bulbul and other remaining native birds in Fiji forests was also suspected based on reported interspecific aggressive interactions towards at least four native species mostly during the bulbul's breeding season. This caused habitat shifts in native birds (Watling 1978, 1983). Thibault et al. (2018d) provide evidence for competition in man-modified habitats in New Caledonia showing a negative relationship between red-vented bulbul and the abundance of nine native (including endemic) species with which its distribution range overlaps, hence red-vented bulbul is believed to drive reassembly of native species toward sub-optimal locations along an urban-rural gradient, by its aggressive behaviour enabling it to out-compete native species and dominate access to food (Thibault et al. 2018d). Interestingly, the abundance of other introduced alien species (*Acridotheres tristis, Passer domesticus, Spilopelia chinensis*) was not affected.

3: Hybridisation

Also, some studies report the presence of hybrids due to the presence of hybrids due to cross-breeding with native related bulbuls, such as white-cheeked bulbul (*Pycnonotus leucogenys*), white-eared bulbul (*P. leucotis*) and yellow-vented bulbul (*P. xanthopygos*) in the middle East (Khamis, 2010; Thibault et al., 2018a8a). In Bahrain, two local bird ringers (Brendan Kavanagh and Abdulla Al-

Khaabi) recorded a cross-breeding incident between the red-vented bulbul and the white-cheeked bulbul (*P. leucogenys*), where they observed hybrid chicks in a nest. A culturally important species, the local population of the white-cheeked bulbul is under the continuous threat of habitat degradation and poaching. Hence, cross-breeding, which leads to sterile offspring, forms an additional threat to this already vulnerable species and should be considered seriously (Khamis, 2010). Hybridisation with common bulbul (*P. barbatus*), which is one of the commonest birds in Africa that only recently started breeding in southern Spain around Tarifa, has not been reported.

4: Predation

Other impacts include predation on invertebrates such as Hemiptera, Coleoptera, Odonata, Lepidoptera (Fox, 2011)), Hymenoptera and Diptera, as well as small reptiles including geckos and skinks (Brooks et al., 2013; Thibault et al., 2019). On Hawaii, predation by red-vented bulbul and red-whiskered bulbul on larvae and adults of the iconic monarch butterfly *Danaus plexippus* (Lepidoptera: Nymphalidae) led to changes in the proportions of colour morphs in the population (Stimson and Kasuya 2000). Clearly, bulbuls are not deterred by the cardiac glycosides in the monarchs' tissues and can exert heavy predation on larvae feeding on their host plant milkweed (Stimson and Berman 1990). However, we could find no evidence of population level impacts on these species.

Based on and in line with these assessments of alien birds and with species-specific studies we consider the current impact of red-vented bulbul outside the risk assessment area as moderate. Although it is noted that its harmful effects on agricultural systems and native fauna could be highly context-dependent (Thibault, 2018a), there is good evidence of the species altering ecosystems through the spread of seeds of other alien and invasive plant species, and for competition with other bird species, but there is no evidence of red-vented bulbul causing extinctions. This is in line with Thibault et al. (2018d) who suggest that red-vented bulbul causes niche contractions rather than mortality in native species, especially in human-modified landscapes where native birds are already under pressure. No documented cases of recovery of native species after *Pycnonotus* eradication was found, but presumably, as native species are rather displaced and pushed out of optimal habitat, this process is also reversible. Hence a moderate impact score but with high confidence as there are a good number of reliable impact studies on red-vented bulbul from several populations in its invasive range.

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	**minimal** minor moderate major	**CONFIDENCE**	**low** medium high

	massive		

Comment:

No direct evidence is available on impact of red-vented bulbul in the risk assessment area. Since the only established population in Spain is presumably still small, impact can be assumed to be minimal but with low confidence due to the lack of impact studies in the RA area.

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

Comment:

The species could have impacts on native biodiversity at places where it becomes more widely established and numerous, such as in peri-urban habitats, but also more natural vegetations like maquis, open, dry scrubland and forest edge habitat. Here, following the same categorisation of impact mechanisms in the alien range (see Qu. 5.1), we identify a number of potentially sensitive receptors (species, habitats, protected areas) within the risk assessment area which could be impacted upon, considering the areas where the species is already established or areas predicted suitable for establishment (Iberia, Mediterranean, Mediterranean islands).

1: Frugivory (spreading the fruit/seeds of alien plants)

Because of its frugivorous diet, the red-vented bulbul is a possible disperser of invasive alien plant seeds (see Q5.1), and could thus facilitate invasions of invasive plants (MacFarlane et al., 2012; Traveset, 2006; Traveset and Richardson 2014). It is well known that island ecosystems are especially sensitive to the impacts of invasive alien species and because of high levels of endemicity (e.g. Tershy et al. 2015, Bellard et al. 2016). The Mediterranean basin is particularly vulnerable to invasive alien plant invasions because its climatic conditions potentially allow the establishment of sub-tropical and tropical species (Lambdon et al. 2008; Brunel et al. 2010; Brundu 2015). Mediterranean islands are especially vulnerable (Lloret et al. 2005; Vila et al. 2006, 2008; Hulme et al. 2008). A number of established and emerging invasive alien plants for Mediterranean countries produce fleshy fruits and could therefore potentially be spread by birds such as the red-vented bulbul in the risk assessment area (cf. Gosper et al. 2005; Spotswood et al. 2012, 2013). American pokeweed (*Phytolacca americana*) and Indian pokeweed (*P. acinosa*) produce fleshy purple berries that are spread by birds (McDonnell et al. 1984). Other examples of (potentially) invasive plants that might be spread by red-vented bulbuls in the risk assessment area include Brazilian peppertree (*Schinus terebinthifolius*), a commonly planted ornamental in the Mediterranean which is reported to be spread by the species (Thibault et al. 2018c), silver-leaved nightshade (*Solanum elaeagnifolium*), a common agricultural weed (Brunel et al. 2010), Sticky nightshade (*S. sisymbriifolium*), a weed of pastures and irrigated crops, roseleaf bramble (*Rubus rosifolius*), Chinese privet (*Ligustrum sinense*) and garden privet (*L. ovalifolium*) (Tanner 2017). Some of these species typically occur in human-modified areas such as parks, gardens and ruderal terrains. By comparison, also red-whiskered bulbuls (*P. jocosus*), a species with comparable ecology, are notorious for spreading invasive weeds including *Lantana* spp., *Rubus* spp., *Phytolacca* spp., *Chrysanthemoides* spp. and *Ligustrum* spp. in mediterranean parts of Australia and this is considered their biggest impact on ecosystems (Mo 2015). In Spain, red-whiskered bulbuls have been reported feeding on seeds and fruits of kurrajong (*Brachychiton populneus*), fig (*Ficus carica*), strawberry tree (*Arbutus unedo*), loquat (*Eriobotrya japonica*), privet (*L. vulgare*), chinaberry tree (*Melia azedarach*), pomegranate (*Punica granatum*), Peruvian pepper (*Schinus molle*), date palm (*Phoenix dactylifera*), oleander (*Nerium oleander*) flowers, feijoa (*Acca sellowiana*) and yucca (*Yucca* sp.). On Mauritius, red-whiskered bulbuls also have similar species in their diets e.g. *Ligustrum robustum*, *Rubus rosifolius*, *Rubus alceifolius* (Linnebjerg et al. 2010). Spread of typical garden ornamentals by red-vented bulbul could be an issue in the Mediterranean considering the habitat preference of the species for gardens and man-made habitat, for example *Trachycarpus fortunei*, *Mahonia aquifolium*, Exotic *Ribes* sp., *Parthenocissus* sp., *Cotoneaster* sp., *Rosa* sp., *Elaeagnus* sp., *Ziziphus jujuba*, *Morus* sp., *Pittosporum* sp., *Myoporum* sp., *Mirabilis jalapa*, *Opuntia* sp., *Lycium* sp., *Lonicera* sp., *Aralia* sp. and *Hedera* sp. With regards to IAS of Union concern (Union list as it stands in 2019), *Persicaria perfoliata* is the only species that produces berry-like fruits (personal communication G. Brundu, 23/10/2019).

2: Competition

Impact on native bird species will mostly occur through competition for food or space including harassment of native birds by (groups of) red-vented bulbul, being chased away or on the nest through territorial interactions (see Qu. 5.1). However, as the species mostly occurs in urban, human influenced landscapes, most of the passerines it would compete with are relatively common species (e.g. blackcap *Sylvia atricapilla*, Sardinian warbler *S. melanocephala*, common blackbird *Turdus merula*, house sparrow *Passer domesticus*) and the presence of the bulbul is expected to lead mostly to niche contraction rather than declines or extinctions (cf. the reported impact of the species on Tahiti monarch, Blanvillain et al., 2003) hence a score of moderate. Also, some species that are highly valued such as Iberian (azure winged) magpie (*Cyanopica cooki*) might be impacted (see Q 5.7).

3: Hybridisation

Hybridisation with common bulbul (*P. barbatus*) could potentially occur in the risk assessment area. However, hybrids have not yet been reported. Also, common bulbul is one of the commonest birds in Africa and it only recently expanded its range and started breeding in southern Spain around Tarifa so this risk is currently limited.

4: Predation

Red-vented bulbul is known to be a predator of insects and smaller (or juvenile) vertebrate prey like geckos and lizards (Thibault et al. 2018c). Using Speybroeck et al. (2016) and data compiled on native reptiles on mediterranean islands Ficetola et al. (2014) we compiled a list of lizards and geckos that could potentially be predated upon. A lot of those have restricted, endemic ranges within mediterranean islands. These include: Greek Algyroides (*Algyroides moreoticus*) (endemic), Dalmatian Algyroides (*A. nigropunctatus*) and Peloponnese slow worm (*Anguis cephallonica*) on Ionian islands; Sicilian wall lizard (*Podarcis waglerianus*) (endemic) on Sicily and the threatened Aeolian wall lizard (*Podarcis raffoneae*) (endemic) on some smaller Aeolian islets; Milos wall lizard (*Podarcis milensis*) (endemic), Balkan green lizard (*Lacerta trilineata hansschweizeri*) (endemic subspecies), Skyros wall lizard (*Podarcis gaigeae*) (endemic) and Erhard's wall lizard (*Podarcis erhardii*) on the Aegean islands; Cretan wall lizard (*Podarcis cretensis*) (endemic), Kotschy's gecko (*Mediodactylus kotschyi bartoni*) (endemic subspecies), Balkan green lizard (*L. trilineata polylepidota*) (endemic subspecies) and Pori wall lizard (*Podarcis levendis*) (endemic) on Crete; Pygmy algyroides (*A. fitzingeri*) (endemic), Tyrrhenian wall lizard (*Podarcis tiliguerta*) (endemic) and European leaf-toad gecko (*Euleptes europaea*) (endemic) on the Thyrrenian islands; Ibiza wall lizard (*Podarcis pityusensis*) (endemic) and Lilford's wall lizard (*Podarcis lilfordi*) (endemic) on the Balearic islands; Kotschy's gecko (*Mediodactylus kotschyi*) on Cyprus and the eastern mediterranean; the more widespread Moorish gecko (*Tarentola mauritanica*) and Turkish gecko (*Hemidactylus turcicus*) across the Mediterranean. Likewise, the list of insects (Lepidoptera, Coleoptera, Orthoptera) that could be predated upon by bulbuls is very long. Considering documented predation on monarch butterfly (*Danaus plexippus*) on Hawaii (see Q 5.1), this species could also be impacted in southern Spain where it is established yet rare (Gil 2006; Lafranchis 2004). Also similar species of Nymphalidae (and other families such as Papilionidae) with conspicuous colours and/or conspicuous larvae could be impacted in Spain and the Mediterranean, such as African queen (*Danaus chrysippus*) but documented information is lacking for the risk assessment area.

However, although in theory population declines of native insects and reptiles are possible, it is unlikely predation on (mostly nocturnal) geckos or lizards would effectively lead to species extinctions and there are no documented examples of extinctions caused by red-vented bulbuls elsewhere in its invasive range. Hence, score is moderate but with low confidence because of lack of specific impact studies relating to the risk assessment area.

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal **minor** moderate major massive	**CONFIDENCE**	**low** medium high

Comment:

We found no documented impact information for the population around Valencia, which is breeding in suburban gardens and sympatric with the more widely established red-whiskered bulbul (*P. jocosus*) (pers. comm. Carlos Gutiérrez-Expósito, 2018), nor the individuals in Torremolinos, Málaga (Spain) or the population on Fuerteventura which established around 2000. Currently, in the risk assessment area, there is no evidence that red-vented bulbul occurs or is spreading in high conservation value habitats. As no studies have been conducted on this subject, the confidence on this response is low, however it is generally difficult to provide proof of impact in early invasion stages.

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	low **medium** high

Comment:

Based on what is known of its ecological amplitude and habitat characteristics, the red-vented bulbul could potentially establish and spread in a variety of habitats, mostly (peri)urban habitats as is the case in Torremolinos (Malagá) and in Valencia where the species frequents gardens in family groups. Here, impact (e.g. through competition) would occur on rather common species (see Q 5.3). However, if the species becomes more widespread, also conservation value habitats could be invaded where the bulbuls could affect species of concern. Habitats protected by the Habitats Directive which could potentially be invaded and impacted upon through seed dispersal of invasive alien plants include sub-Mediterranean and temperate scrub, Mediterranean arborescent matorral (maquis), Mediterranean sclerophyllous forests (e.g. wild olive woodland, cork-oak forests), garrigue (also known as phrygana in the eastern Mediterranean) and maquis shrubland, which is a complex of several possible vegetation types but characterized by densely growing evergreen shrubs. Several of these vegetation types have unique representation as specific habitats of the Habitats Directive on islands in the Mediterranean (e.g. Tyrrhenian islands, Ionian islands, Cyprus, Malta) (based on European Commission 2013). The Mediterranean scrub biome is also home to a number of breeding birds that could be affected by the red-vented bulbul through competition for nesting space and food. These include a range of songbirds (shrikes, warblers, buntings etc.) and include many species protected by the Birds Directive as well as species listed as vulnerable on the IUCN Red List e.g. Iberian grey shrike (*Lanius meridionalis meridionalis Temminck, 1820*), bunting species such as ortolan bunting (*Emberiza hortulana*) or commoner species of similar habitats like European stonechat (*Saxicola rubicola*). Currently, the red-vented bulbul is present in Valencia (established) and Torremolinos (not established but status remains unclear) (Spain), both areas with typical Mediterranean vegetation that fall within the modelled distribution area for the species. The vulnerable species listed above are also present in that area. Other vertebrate species that could potentially be affected include geckos and lizards, many of which are endemic or have very restricted ranges and are listed on the Annexes II and IV of the EU Habitat Directive.

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	low **medium** high

Comment:

The red-vented bulbul is a known pest species on horticultural and agricultural produce outside its native range, e.g. in plant nurseries or on fruits and vegetables (see Qu. 5.9). It has become a pest in agriculture and an active disperser of invasive alien plants (Shine et al., 2003). In 1999, Decree N°171CM, prepared by the Délégation à l’Environnement listed red-vented bulbul among 3 other alien birds as a threat to biodiversity (Shine et al., 2003).

1 / Provisioning ecosystem services: The species may have an impact on provisioning ecosystem services such as cultivated terrestrial plants grown for nutritional purposes and as ornamentals.

2 / Cultural ecosystem services: Impacts on cultural ecosystem services may include disturbance of the heritage of isolated island ecosystems in case red-vented bulbul establishment and spread comes at the expense of endemic species. This could occur through changes in abundance of native bird species

driving a spatial reassembly of the avifauna. Also, this would especially occur when the species would cause changes in vegetation by promoting seed dispersal of unwanted invasive alien plant species, which could alter ecosystem structure and species composition and make landscapes less attractive for recreation and wildlife watching, or impact the qualities of ecosystems with cultural importance.

These impacts are mostly documented on Pacific islands, not in other areas. In addition, no studies have addressed ecosystem services impact specifically, but there is evidence of economic and ecosystem impact, hence we score confidence as medium.

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	**minimal** minor moderate major massive	**CONFIDENCE**	**low** medium high

Comment:

No information has been found on ecosystem services impact of the red-vented bulbul where it established in the RA area (Spain). The population in Valencia is breeding in gardens of urbanized areas surrounding the city (personal communication Carlos Gutiérrez-Expósito, 2018).

1 / Provisioning ecosystem services: there may be impacts on provisioning ecosystem services by damage to plants, fruits and legumes grown in gardens or commercial produce (e.g. citrus and tomato around Valencia) but this would probably be very localized (see Q 5.11).

2 / Cultural ecosystem services: impacted species could include the Iberian magpie (*Cyanopica cooki*), Iberian grey shrike (*Lanius meridionalis*) and ortolan bunting (*Emberiza hortulana*). Iberian magpies are a typical element of Iberian avifauna and have been split from their Asian conspecific based on genetic evidence (Kryukov et al. 2004). The species is highly valued by birdwatchers. Iberian magpies roam in groups in open woodland with grassy clearings, including orchards and olive groves. Their diet consists mainly of acorns and pine nuts, supplemented by invertebrates, soft fruits and berries, and also human-provided scraps in parks and towns. Clearly, they are in the same feeding niche as red-vented bulbul. Iberian magpies roam maritime pine (*Pinus pinaster*) forests, a protected European Habitat (Annex I habitat type 2270 - wooded dunes with *P. pinea* and/or *P. pinaster*), and also dehesa (Annex I habitat type 6310 - Dehesas with evergreen *Quercus* spp), open parklands of *Quercus ilex rotundifolia* used for cattle grazing and a well-known traditional, culturally highly valued landscape in rural Iberia. A large proportion of the surface area (35.3%) of this typically Mediterranean agrosilvopastoral ecosystem is classified by UNESCO as a Biosphere Reserve and it is also part of the Natura 2000 network of protected areas (Massot 2016). The presence of large populations of bulbuls, which often flock together in noisy family groups, could cause changes in such valued native bird

assemblages, including in parks and gardens where people go to appreciate native wildlife. Other examples of common species include warblers like Eurasian blackcap (*Sylvia atricapilla*), Sardinian warbler (*S. melanocephala*) or thrushes such as common blackbird (*Turdus merula*) or song thrush (*T. philomelos*).

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate **major** massive	**CONFIDENCE**	low **medium** high

Comment:

Within the Mediterranean biogeographic region, the red-vented bulbul will impact on several ecosystem services, including:

- Provisioning - Biomass - Cultivated terrestrial plants - Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes
- Regulation & Maintenance - Regulation of physical, chemical, biological conditions - Lifecycle maintenance, habitat and gene pool protection - Seed dispersal
- Cultural - Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting - Physical and experiential interactions with the natural environment - Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions
- Cultural - Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting - Physical and experiential interactions with the natural environment - Intellectual and representative interactions with natural environment - Characteristics of living systems that enable aesthetic experiences
- Cultural - Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting - Other biotic characteristics that have a non-use value - Characteristics or features of living systems that have an option or bequest value

The red-vented bulbul has a diverse diet including fruits, berries, flowers and buds (Islam and Williams, 2000; Brooks, 2013; Vander Velde, 2002). Birds can feed on unripe fruits and buds in large flocks (Fox, 2011), and as a consequence, damage to cultivated plants is the most frequently reported impact of the red-vented bulbul in its alien range. However, these studies were conducted in a limited number of places, such as on Hawaii and in New Caledonia (Thibault et al., 2018b). In a global literature review, Thibault et al. (2018c) report damage to at least 52 plant species belonging to 25 families with 67% (35 species) being food plants (fruits and legumes such as papayas, bananas, lychee, mangos, spinach, cucumber, courgette, aubergine, dragon fruit) and 33% (17 species) being ornamental plant species (e.g. orchids, *Hibiscus* spp.). These numbers are underestimations as many reports of consumption by red-vented bulbul do not consider the type of impact (damage to production or seed dispersal). The impact of the red-vented bulbul appears to be particularly serious on Oahu (Hawaï), where the birds are reported consuming several species of fruits, vegetables and flowers, leading to considerable economic losses. In New Caledonia, significant impacts have been recorded for some crops and plant nurseries with up to 35% losses (Caplong and Barjon, 2010). Significant impacts were observed on production fruit trees, with no less than 35% loss in attacked orchards. Damage was recorded on lychee and peach production but also on papaya and other fruits, sometimes up to the total destruction of the orchard (Metzdorf and Brescia, 2008). Moreover, damage to red fruits in general (tomatoes, strawberries) was reported with losses on tomato production of 500 kg per week as is damage to buds and flowers (Metzdorf and Brescia, 2008). Damage to aubergine crops has also been reported, but also other crops such as dragon fruit (*Pitaya sp.*) (Thouzeau-Fonseca, 2013). Conversely, the red-vented bulbul is not considered an agricultural pest in Fiji (Watling, 1979), nor in Houston (Texas, USA) where it was found to consume mainly introduced tropical plant species (Brooks, 2013).

Next, to crop or ornamental plant damage, the red-vented bulbul also impacts seed dispersal, as it mainly acts as a vector for seeds of invasive plant species. For instance, a study conducted by Spotswood et al. (2013) in French Polynesia showed that the red-vented bulbul prefers the fruit of a highly invasive tree (*Miconia calvescens*) over that of three other species (one alien, two native). Also in New Caledonia, the red-vented bulbul showed preference for non-native fruit species, including the highly invasive *S. terebinthifolius* (Thibault et al., 2018b). In addition, gut transition led to enhanced germination rates and could represent an “invasional meltdown”, a mutualistic relationship between invasive seed dispersers and invasive plant species leading to higher numbers/faster spread rates of both species and possibly major conservation issues, particularly in ecosystems that host a large number of endemic plant species (Thibault et al., 2018b).

Lastly, since the red-vented bulbul could have an impact on the distribution of less territorial native species, it could alter the species composition in the invasive range, impacting on several cultural ecosystem services as listed above.

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate **major** massive	**CONFIDENCE**	low **medium** high

Comment:

As stated in Qu. 5.8, the red-vented bulbul is considered an agricultural pest species in several parts of its invasive range. The estimated value of the damage to Oahu's Orchid industry in one 1989 was $300,000 (Fox, 2011) when the red-vented bulbul together with the Japanese white-eye (*Zosterops japonicus*) reportedly destroying up to 75% of Hawaiian *Dendrobium* orchid and *Anthurium* plantations. This also prompted investigations into chemical repellents to keep the birds off orchid plantations (Cummings et al., 1994). Also in New Caledonia, Thibault et al. (2019) report an economic loss of approximately $18,355 USD for September 2016 alone in tomato plots.

The species is a well-known agricultural pest species in many parts of the world and there are many, although mostly anecdotal, records of economic damages. Many more probably remain unreported, so we assume yearly damages can easily mount up to more than 1,000,000 euro and scored major.

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found". Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**minimal** minor moderate major massive	**CONFIDENCE**	**low** medium high

Comments:

No information has been found on the issue.

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	minimal	**CONFIDENCE**	low

	minor **moderate** major massive		**medium** high

Comments:

Although Thibault et al. (2019) report considerable economic damages outside the native range (e.g. on tomato production, *Hibiscus* and orchids) (see Qu 5.8 and 5.9), Brooks (2013) reports no damage of that kind in Houston and states that flock sizes remain small (average 2.3 birds/flock, range 1–22) compared to the native range which also limits the potential for damage. Also, although this would involve extra costs, bulbul damage can be mitigated using nets, repellents or other methods (see Annex IV). Spain is an important producer of fresh fruit and vegetables which are mostly exported to other EU Member States (https://wits.worldbank.org/), with the area of Valencia where the species is currently established as an important citrus region. Tomatoes are also of great economic importance in Spain, as it is the world's 8th producer, with a production of 5,163 million kilos, grown on an area of 60,852 hectares (www.hortoinfo.es). In Torremolinos, Malagá, the main crops of economic importance that could potentially be impacted include almonds, sunflowers and olives (Massot 2016), apart from small agricultural produce of vegetables in gardens. In case red-vented bulbul establishes more widely in the risk assessment area, economic damages could occur, however, they would probably be localized and context dependent. Also, methods are available to mitigate or prevent bird damages to sensitive crops (e.g. Tracey et al. 2007).

Another vulnerable receptor is the orchid industry, but damage to the orchid industry seems unlikely, given that most orchids in the risk assessment area are produced in The Netherlands in greenhouses, which is outside of the predicted distribution area of the red-vented bulbul and greenhouses are not easily accessible to birds.

In line with other examples of damages outside the native range, but considering the importance of vegetable production in the Mediterranean, we scored moderate.

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	**minimal** minor moderate major massive	**CONFIDENCE**	low medium **high**

Comments:

The species is currently not under management in the risk assessment area, therefore we can say with confidence the current management cost is minimal.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	**low** medium high

Comments:

There are examples of bulbul eradications on islands using a variety of methods (mostly mist netting with or without supplementary shooting, see Annex IV) (Bunbury et al., 2019, Saavedra & Reynolds, 2019, Bunbury et al., 2019; http://diise.islandconservation.org/). On Fuerteventura, 7 birds were caught in 2010 (Saavedra & Reynolds, 2019). However, the costs of such eradication/control efforts are not documented. As a crude proxy, Holmes et al. (2015) provide costings for island eradications of predators, and show that although the implementation costs per ha can be relatively low, the planning phase, isolation and the presence of human habitation (often the case with red-vented bulbul invasions) can add up to great expense. As a comparison, Parkes et al. (2006) estimated that the costs to achieve eradication of common myna (*A. tristis*) from Mangaia (Cooke islands, 5180 ha) with appropriate levels of monitoring would be about NZ$100,000, with 80% of that budget needed for preparation and training and surveillance, including detecting surviving birds. However, here, the method proposed was toxic baiting.

As stated in Qu. 5.11, mitigation of bird damage to produce will also involve additional management costs.

Considering the costs described both above and in Holmes et al. (2015) and assuming the red-vented bulbul would establish more widely in the RA area in suburban mainland areas and/or on islands, we scored moderate but since data on costs from the literature are not species specific, confidence is low.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of

people, property or infrastructure;

- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**minimal** minor moderate major massive	**CONFIDENCE**	**low** medium high

Comments:

No social or human health impact has been caused by the red-vented bulbul so far. However, red-vented bulbuls in Tahiti have been found to carry the zoonotic disease *Chlamydia sp.* (Blanvillain et al., 2013).

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	**minimal** minor moderate major massive	**CONFIDENCE**	**low** medium high

Comments:

See Qu. 5.14. This is not expected to change in the future.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	minimal	**CONFIDENCE**	**low**

	minor moderate major massive		medium high

Comments:

Dispersal of parasites by red-vented bulbul is not well documented in its alien range. In its native range, the red-vented bulbul is known to host *Isospora* spp. (Boughton et al. 1938), *Menacanthus eurysternus* (Price 1975), *Bruelia guldum* and *Sturnidoecus guldum* (Ansari 1957) and *Pteroherpus pycnonoti* (Constantinescu et al., unpublished). In 1996, Jarvi et al. (2003) detected no avian malaria (*Plasmodium* spp.) in blood smears, and Atkinson et al. (2006) found no evidence of *Plasmodium*, *Trypanosoma*, *Atoxoplasma* or microfilaria. *Plasmodium* is however present in the south Pacific area (Martin Thibault, pers. comm.). Red-vented bulbuls in Tahiti, however, have been found to carry the zoonotic disease *Chlamydia* (Blanvillain et al. 2013).

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	**minimal** minor moderate major massive	**CONFIDENCE**	**low** medium high

Comments:

No other possible impacts were found.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	low **medium** high

Comments:

The red-vented bulbul is not particularly vulnerable to predation. In its native range, a study carried out by Zia et al. (2017) found that predation rate on the red-vented bulbul was only 6% in eggs and 9% in nestlings. Predators in the study area included raptor species like eagles and barn owl (*Tyto alba*), and rodents like the black rat *(Rattus rattus*). These species or similar species are also present in the risk assessment area, possibly impacting the red-vented bulbul. However, if these only have minor effects in the native range, it is unlikely that their impact will be higher in the introduced range, where they are not used to hunt on the red-vented bulbul. Likely predators of the red-vented bulbul in Valencia and Torremolinos, and by extension the risk assessment area, include the booted eagle (*Hieraaetus pennatus* Gmelin, 1788), Bonelli's eagle (*Aquila fasciata* Vieillot, 1822), the northern goshawk (*Accipiter gentilis* Linnaeus, 1758) and the sparrowhawk (*Accipiter nisus* Linnaeus, 1758). The latter might become the most important predator of the red-vented bulbul in its invasive range, since it mainly preys upon species similar to the red-vented bulbul regarding size, behaviour and habitat, such as the house sparrow (*Passer domesticus* Linnaeus, 1758), common blackbird (*Turdus merula* Linnaeus, 1758), starlings and pigeons. Other birds of prey may take the eggs of the red-vented bulbul, as will mammalian predators like the stone martin (*Martes foina* Erxleben, 1777) and European pine marten (*Martes martes* Linnaeus, 1758). Since the red-vented bulbul nests in trees, it will not be vulnerable to ground predators such as foxes or stoats.

Although the red-vented bulbul is a known host to several parasites in its native range, including *Isospora* spp. (Boughton et al. 1938), *Menacanthus eurysternus* (Price 1975), *Bruelia guldum* and *Sturnidoecus guldum* (Ansari 1957) and *Pteroherpus pycnonoti* (Constantinescu et al., unpublished), no impacts of these parasites on the red-vented bulbul were found.

No information was found on impact of pathogens on the red-vented bulbul.

For more information, see Qu. 3.5.

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor **moderate** major massive	**CONFIDENCE**	low **medium** high

Response:

See answers to Qu. 5.3, 5.5, 5.8, 5.11, 5.13, 5.15 and 5.16.

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal **minor** moderate major massive	**CONFIDENCE**	low **medium** high

Response:

As stated in Qu. 3.13 and 4.12, establishment potential for the red-vented bulbul will likely be lower in both climate change scenarios. Therefore, we expect impact to be lower as well.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Introduction*	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The species is already present in zoological gardens and private collections in multiple countries within the risk assessment area. There is also the possibility that the species is introduced for private bird collections although quantitative data are lacking. Introductions as stowaways are less likely.
Summarise Entry*	very unlikely unlikely moderately likely likely **very likely**	low **medium** high	The red-vented bulbul is kept as a pet and is on display in zoological garden. Escapes and releases have happened before in several countries in the risk assessment area and entry is likely to occur in the future. Stowaways on ships might enter the risk assessment area as well, as the species is established on the Canary islands.
Summarise Establishment*	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	The species already established on at least two locations in the risk assessment area (Spain). The Mediterranean bioregion, including Mediterranean islands, is generally vulnerable to invasion by red-vented bulbul, both under current and future climatic conditions. The red-vented bulbul is most likely to establish in (peri)urban areas with a mediterranean or (semi-)arid climate.
Summarise Spread*	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	Spread of the red-vented bulbul within the risk assessment The species is classified as a rather sedentary species, showing possible movements depending on environmental conditions. Indeed, spread is limited in Spanish

			mainland populations, although densities are still limited here and the species has to compete with red-whiskered bulbul. However, the invasion on Fuerteventura, where the species invaded the entire island in a few decades and has shown important range expansion in a short period of time, shows red-vented bulbul is able to spread moderately rapidly or rapidly in insular contexts.
Summarise Impact*	minimal minor **moderate** major massive	low **medium** high	The red-vented bulbul can impact on native species and ecosystems through competition, frugivory, by spreading alien plants, hybridising with bulbul species, by predating on (in)vertebrates and by pathogen transmission. It is also an agricultural pest in parts of its alien range. However, although declines and niche contraction in sensitive and protected species are possible, no extinctions caused by red-vented bulbul have been documented so far.
Conclusion of the risk assessment (overall risk)	low **moderate** high	low **medium** high	Considering the potential of red-vented bulbul to cause population declines in native species, documented contractions of niches of native species in its alien range but no extinctions, moderate spread capacity, the limited area suitable for establishment in the risk assessment area but presence of sensitive island biota, local and reversible effects on ecosystem services and the potential for moderate economic damages, we scored moderate impact. This score is in line with a recent, global, environmental impact assessment of alien birds using EICAT.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	-	-	-	-	-
Belgium	Yes	-	-	-	-
Bulgaria	-	-	-	-	-
Croatia	-	-	-	-	-
Cyprus	-	-	Yes	Yes	-
Czech Republic	-	-	-	-	-
Denmark	-	-	-	-	-
Estonia	-	-	-	-	-
Finland	-	-	-	-	-
France	-	-	Yes	Yes	-
Germany	-	-	-	-	-
Greece	-	-	Yes	Yes	-
Hungary	-	-	-	-	-
Ireland	-	-	-	-	-
Italy	-	-	?	?	?
Latvia	-	-	-	-	-
Lithuania	-	-	-	-	-
Luxembourg	-	-	-	-	-
Malta	-	-	?	?	?
Netherlands	Yes	-	-	-	-
Poland	-	-	-	-	-
Portugal	-	-	?	?	?
Romania	-	-	-	-	-
Slovakia	-	-	-	-	-
Slovenia	-	-	-	-	-
Spain	Yes	Yes	Yes	Yes	
Sweden	-	-	-	-	-
United Kingdom	-	-	-	-	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	-	-	-
Atlantic	-	-	-	-	-
Black Sea	-	-	-	-	-
Boreal	-	-	-	-	-
Continental	-	-	-	-	-
Mediterranean	-	-	Yes	Yes	-
Pannonian	-	-	-	-	-
Steppic	-	-	-	-	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea					
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast					
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea					
Aegean-Levantine Sea					
Ionian Sea and the Central Mediterranean Sea					
Western Mediterranean Sea					

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[7]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measurable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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[7] Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition,*

			predations, spread of disease etc.)
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[8]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by microorganisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by microorganisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*

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[8] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*

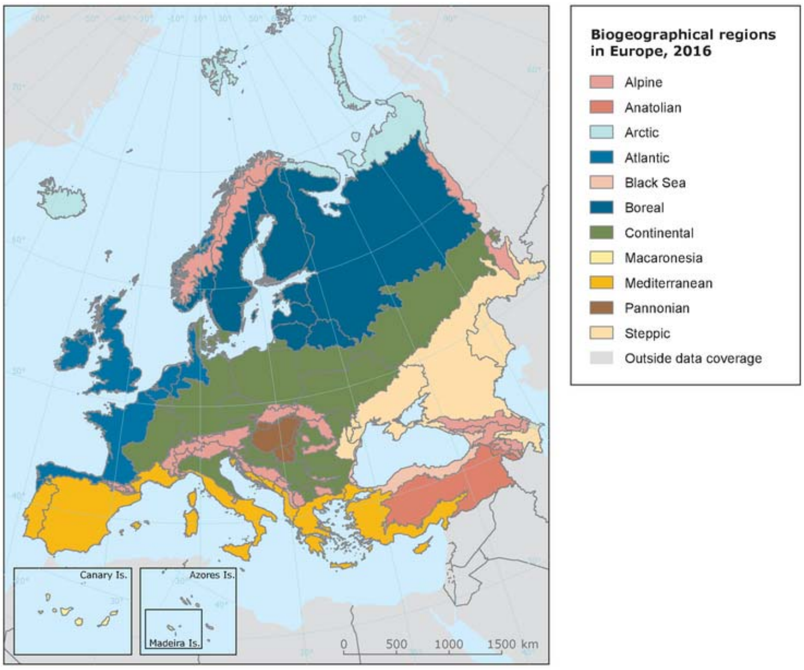
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

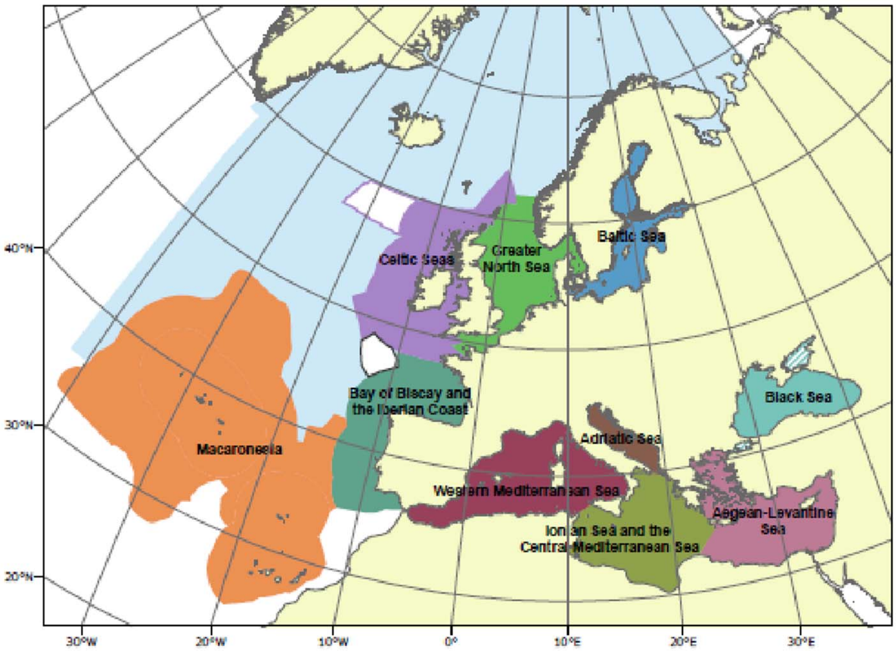
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

ANNEX VII Projection of climatic suitability for *Pycnonotus cafer* establishment

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31 October 2019

Aim

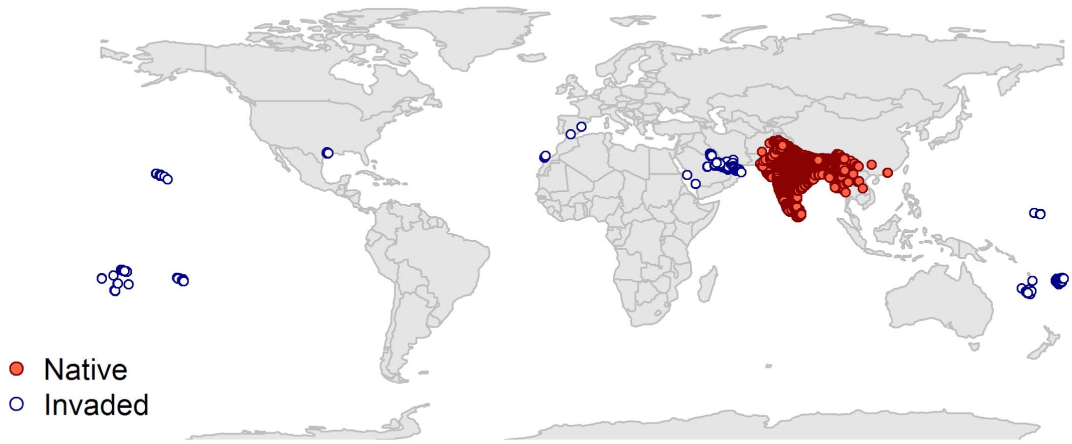
To project the suitability for potential establishment of *Pycnonotus cafer* in Europe, under current and predicted future climatic conditions.

Data for modelling

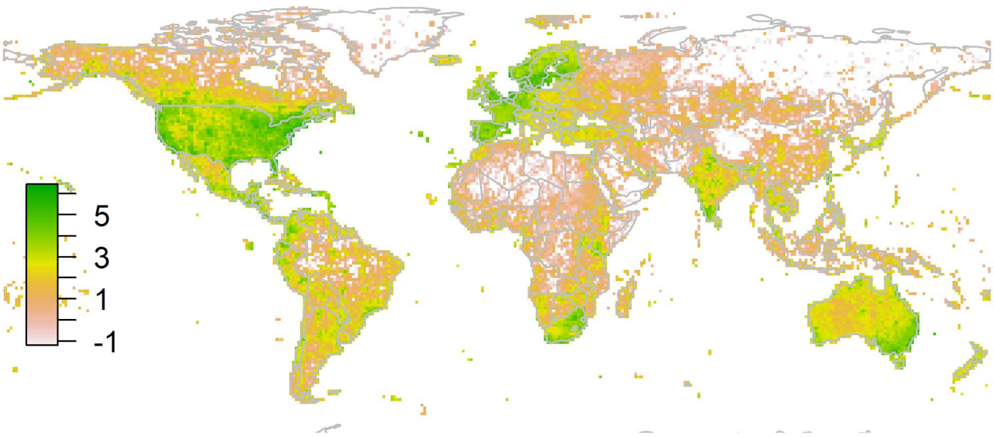
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (245716 records), the Biodiversity Information Serving Our Nation database (BISON) (8660 records), iNaturalist (358 records), the Integrated Digitized Biocollections (iDigBio) (42 records), and a small number of additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils, captive records) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 3381 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Aves records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Pycnonotus cafer* and used in the modelling, showing native and invaded distributions. (b) The recording density of Aves on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the ‘Bioclim’ variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Pycnonotus cafer*, the following climate variables were used in the modelling:

- Annual mean temperature (Bio1)
- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Annual precipitation (Bio12)
- Precipitation of the wettest month (Bio13)
- Precipitation of the driest month (Bio14)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. There represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Tree cover (Tree): This was estimated from the MODerate-resolution Imaging Spectroradiometer (MODIS) satellite continuous tree cover raster product, produced by the Global Land Cover Facility (http://glcf.umd.edu/data/vcf/). The raw product contains the percentage cover by trees in each 0.002083 x 0.002083 degree grid cell. We aggregated this to the mean cover in our 0.25 x 0.25 degree grid cells.
- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.

Species distribution model

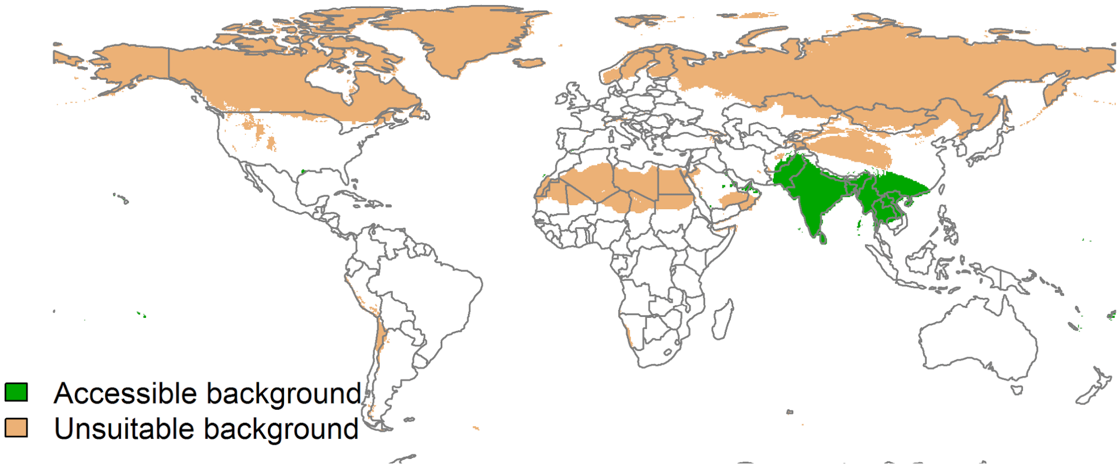
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1 (Thuiller et al., 2019, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Pycnonotus cafer* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 300km buffer around the native range occurrences; AND
- A 50km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Pycnonotus cafer* at the spatial scale of the model:
    - Annual mean temperature (Bio1) < 4
    - Annual precipitation (Bio12) < 4

Altogether, only 0.5% of occurrence grid cells were located in the unsuitable background region.

Within the background region, 10 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Pycnonotus cafer*. Samples were taken from a 300km buffer around the native range and a 50km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCdist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Pycnonotus cafer* was most strongly determined by Annual mean temperature (Bio1), accounting for 42.9% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (23.5%), Precipitation of the wettest month (Bio13) (10.2%), Minimum temperature of the coldest month (Bio6) (9.4%), Human influence index (HII) (5.7%), Annual precipitation (Bio12) (5.3%), Precipitation of the driest month (Bio14) (2.2%) and Global tree cover (Tree) (0.8%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

					variable importance (%)							
Algorithm	AUC	Kappa	TSS	Used in the ensemble	Annual mean temperature (Bio1)	Mean temperature of the warmest quarter (Bio10)	Precipitation of the wettest month (Bio13)	Minimum temperature of the coldest month (Bio6)	Human influence index (HII)	Annual precipitation (Bio12)	Precipitation of the driest month (Bio14)	Global tree cover (Tree)
GLM	0.840	0.488	0.614	yes	60	7	10	9	12	1	1	0
GAM	0.841	0.503	0.616	yes	56	16	7	7	8	4	1	1
ANN	0.847	0.530	0.650	yes	22	31	8	25	5	4	3	2
GBM	0.839	0.512	0.624	yes	42	29	11	4	2	8	3	1
MARS	0.837	0.488	0.614	yes	35	34	16	1	1	11	2	0
RF	0.697	0.478	0.609	no	20	14	13	17	9	10	10	8
Maxent	0.835	0.500	0.613	no	39	34	7	4	7	6	2	2
Ensemble	**0.846**	**0.522**	**0.628**		**43**	**24**	**10**	**9**	**6**	**5**	**2**	**1**

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

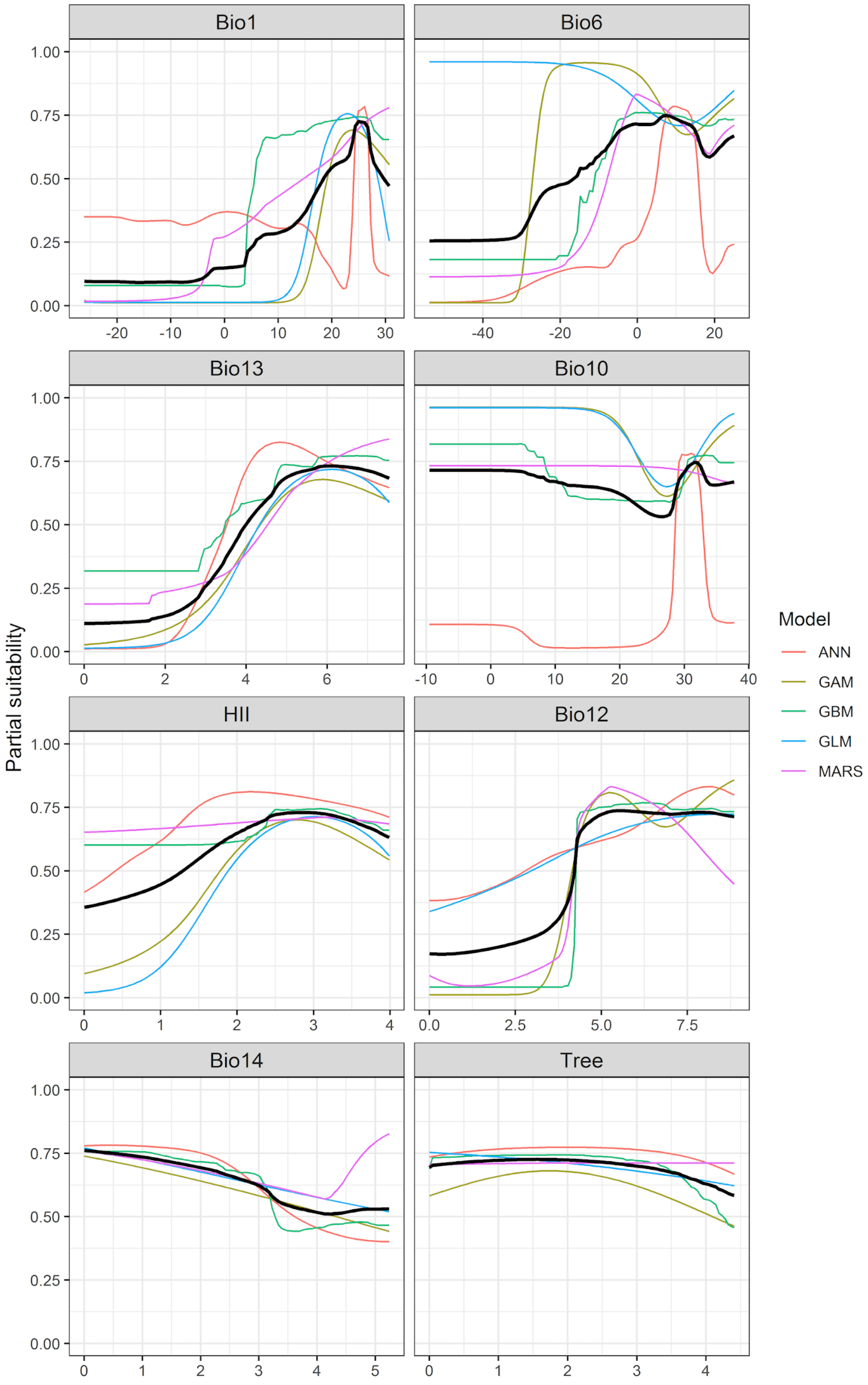
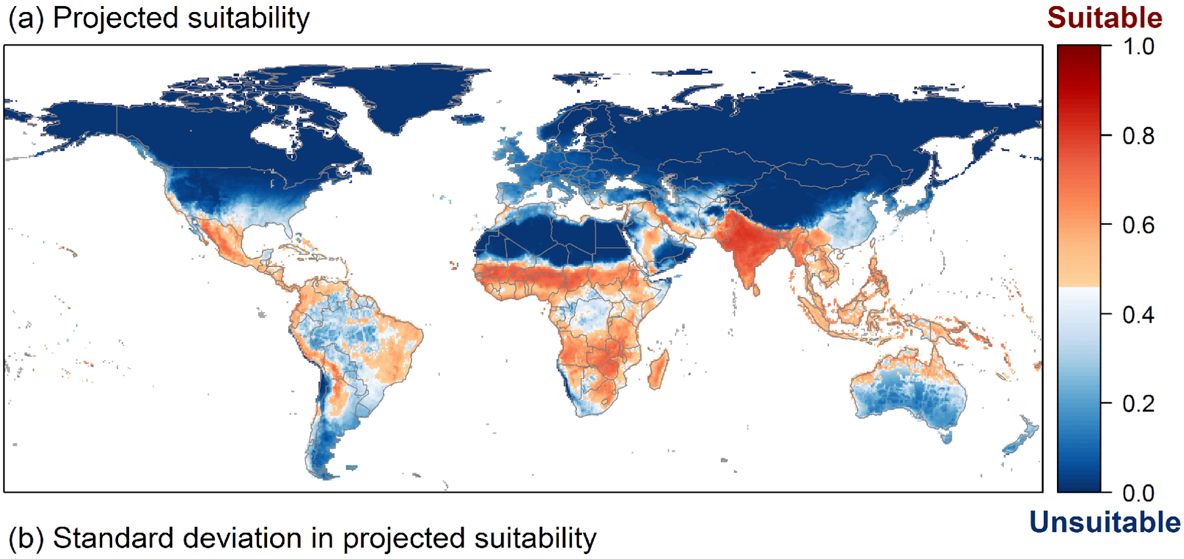


Figure 4. (a) Projected global suitability for *Pycnonotus cafer* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.46 may be suitable for the species. Grey areas have missing data in a predictor layer. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

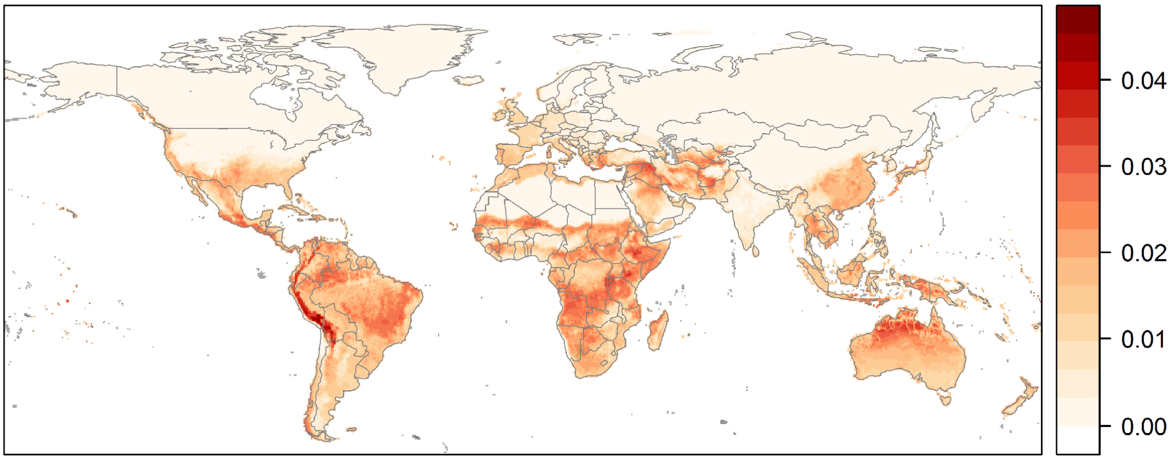
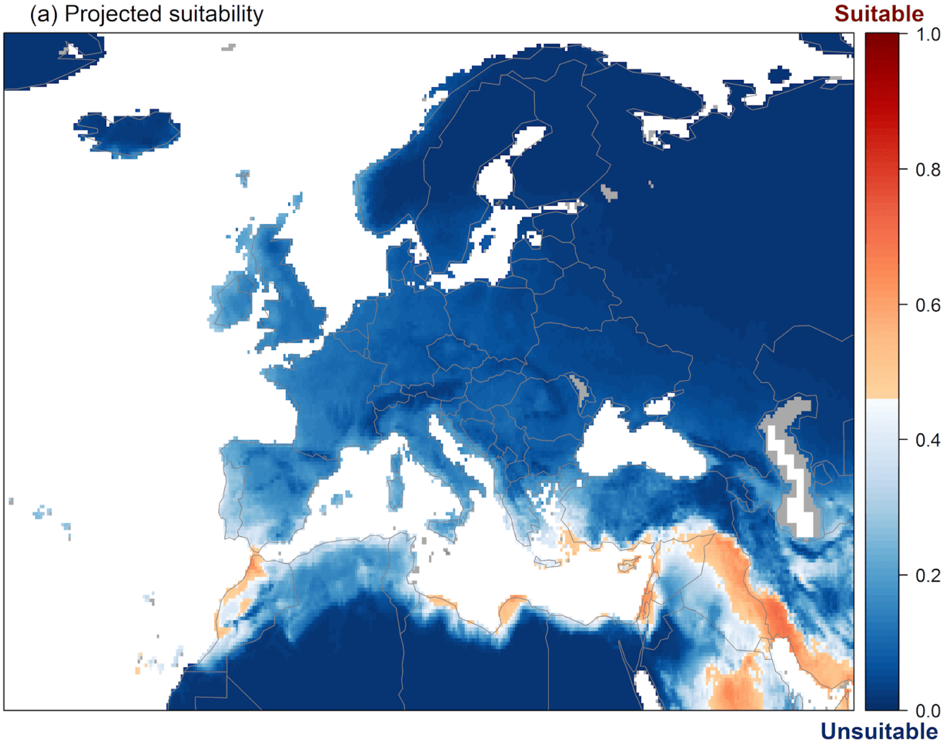


Figure 5. (a) Projected current suitability for *Pycnonotus cafer* establishment in Europe and the Mediterranean region. Grey areas have missing data in a predictor layer. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

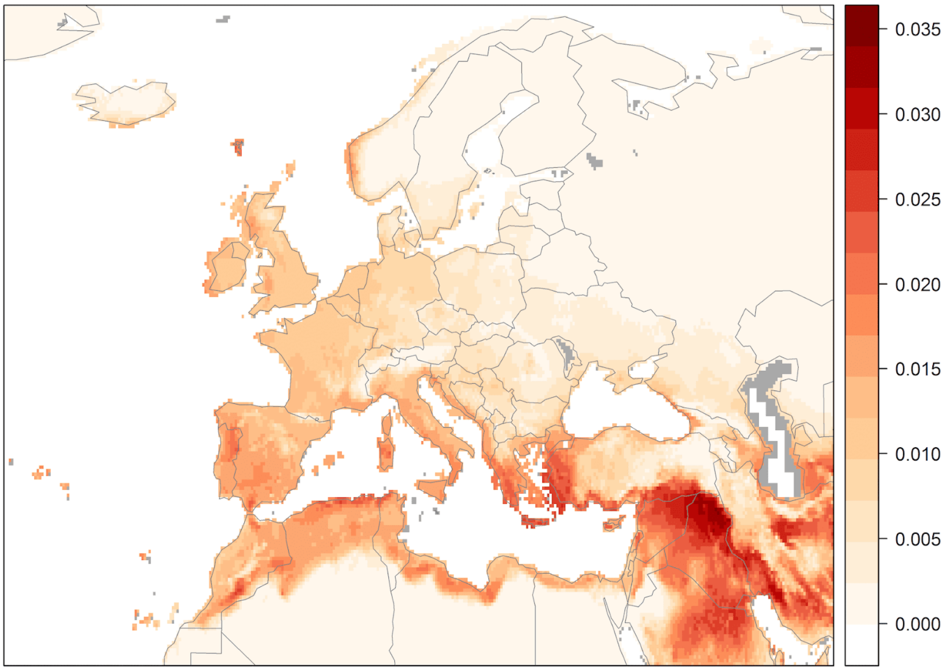


Figure 6. The most strongly limiting factors for *Pycnonotus cafer* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

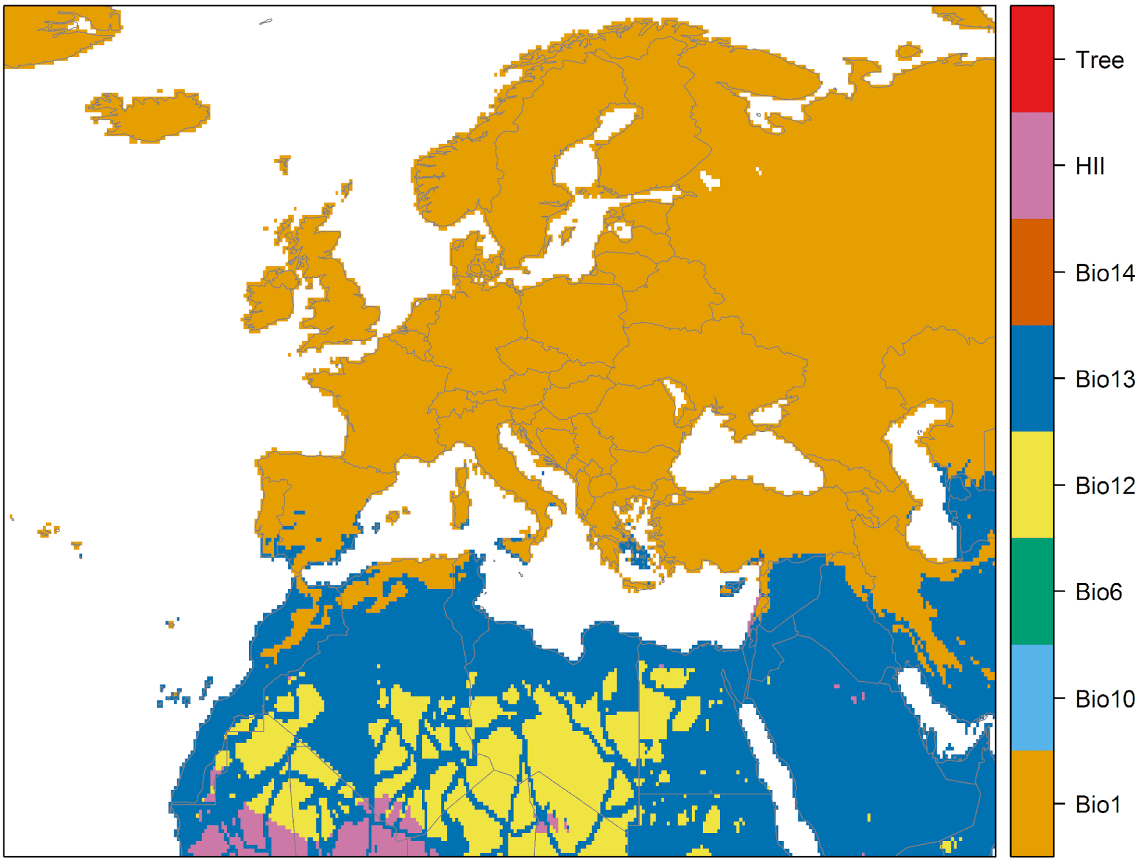
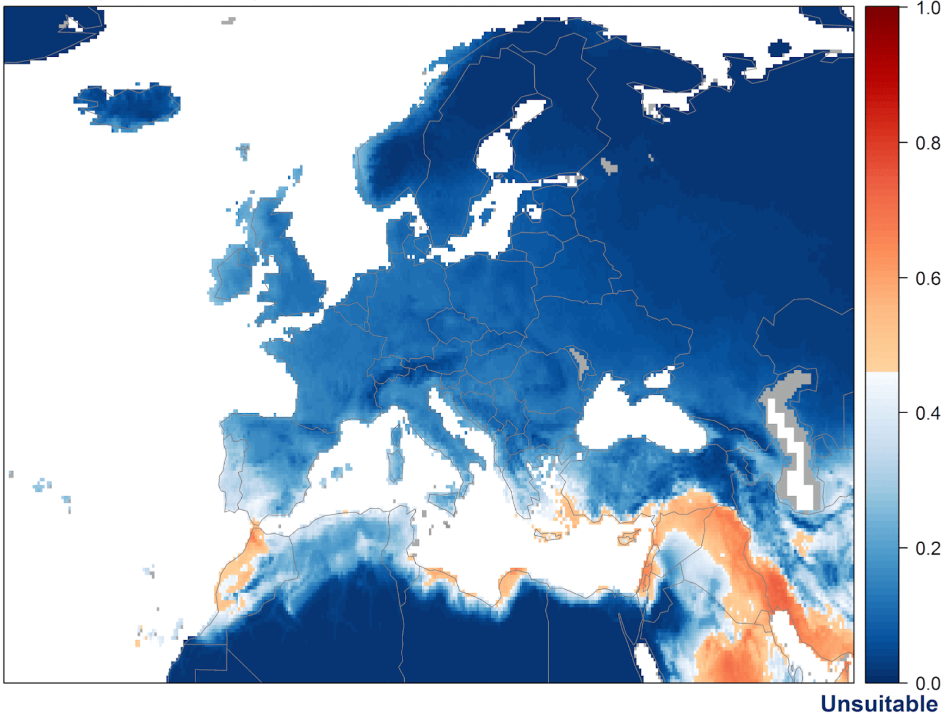


Figure 7. (a) Projected suitability for *Pycnonotus cafer* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. Grey areas have missing data in a predictor layer. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

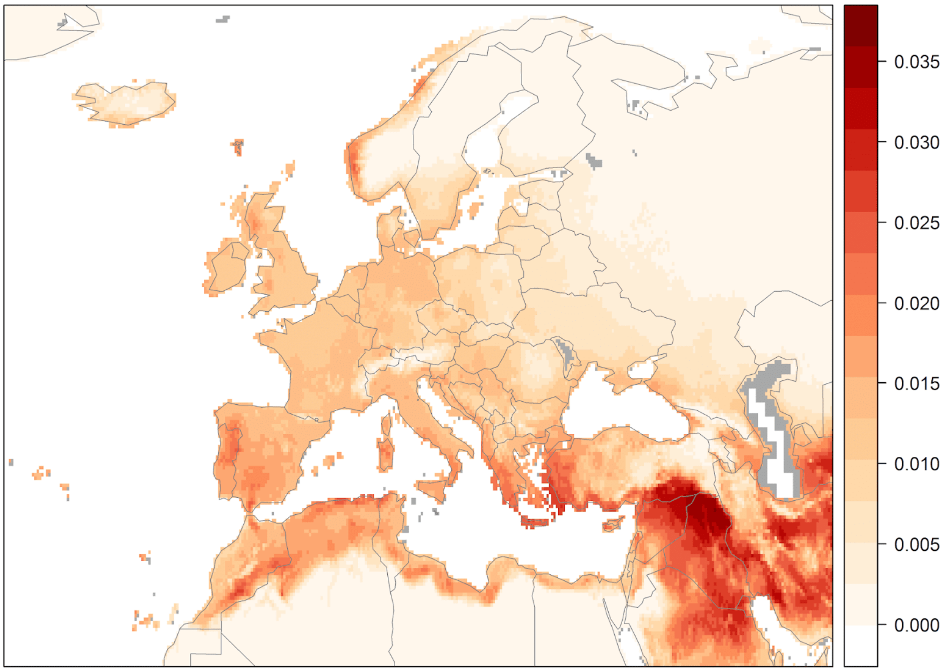
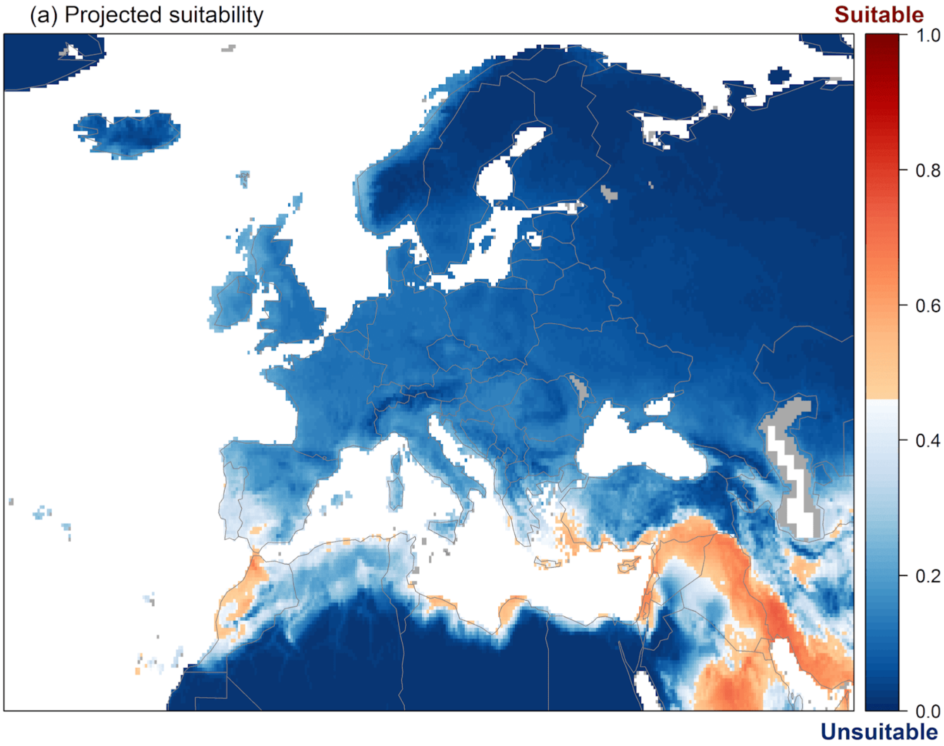


Figure 8. (a) Projected suitability for *Pycnonotus cafer* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. Grey areas have missing data in a predictor layer. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.


(a) Projected suitability

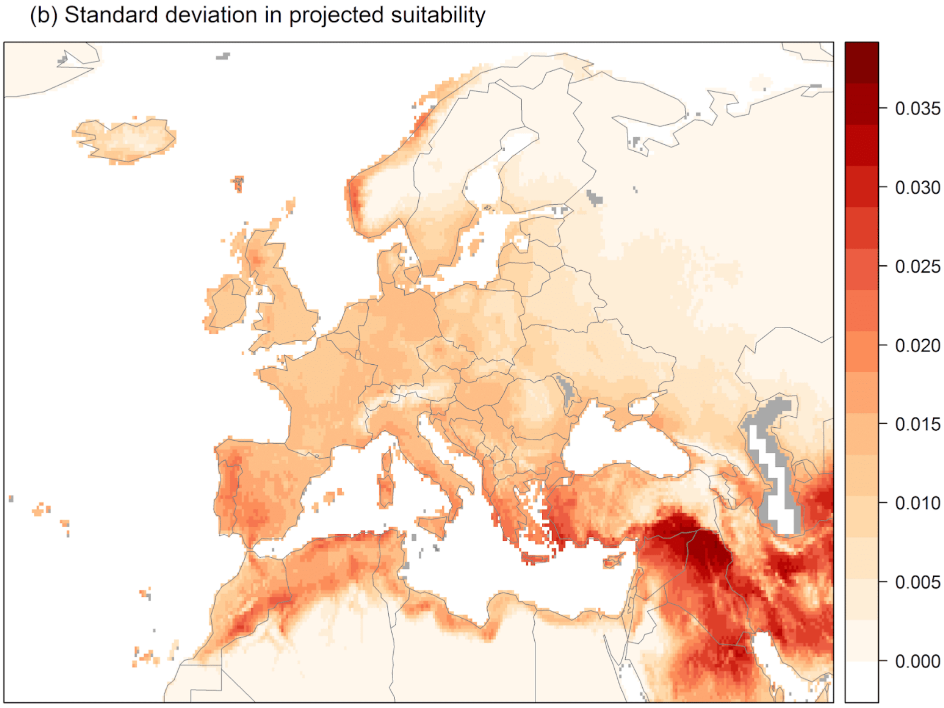

(b) Standard deviation in projected suitability

Figure 9. Variation in projected suitability for *Pycnonotus cafer* establishment among Biogeographical regions of Europe (Bundesamt für Naturschutz (BfN), 2003). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

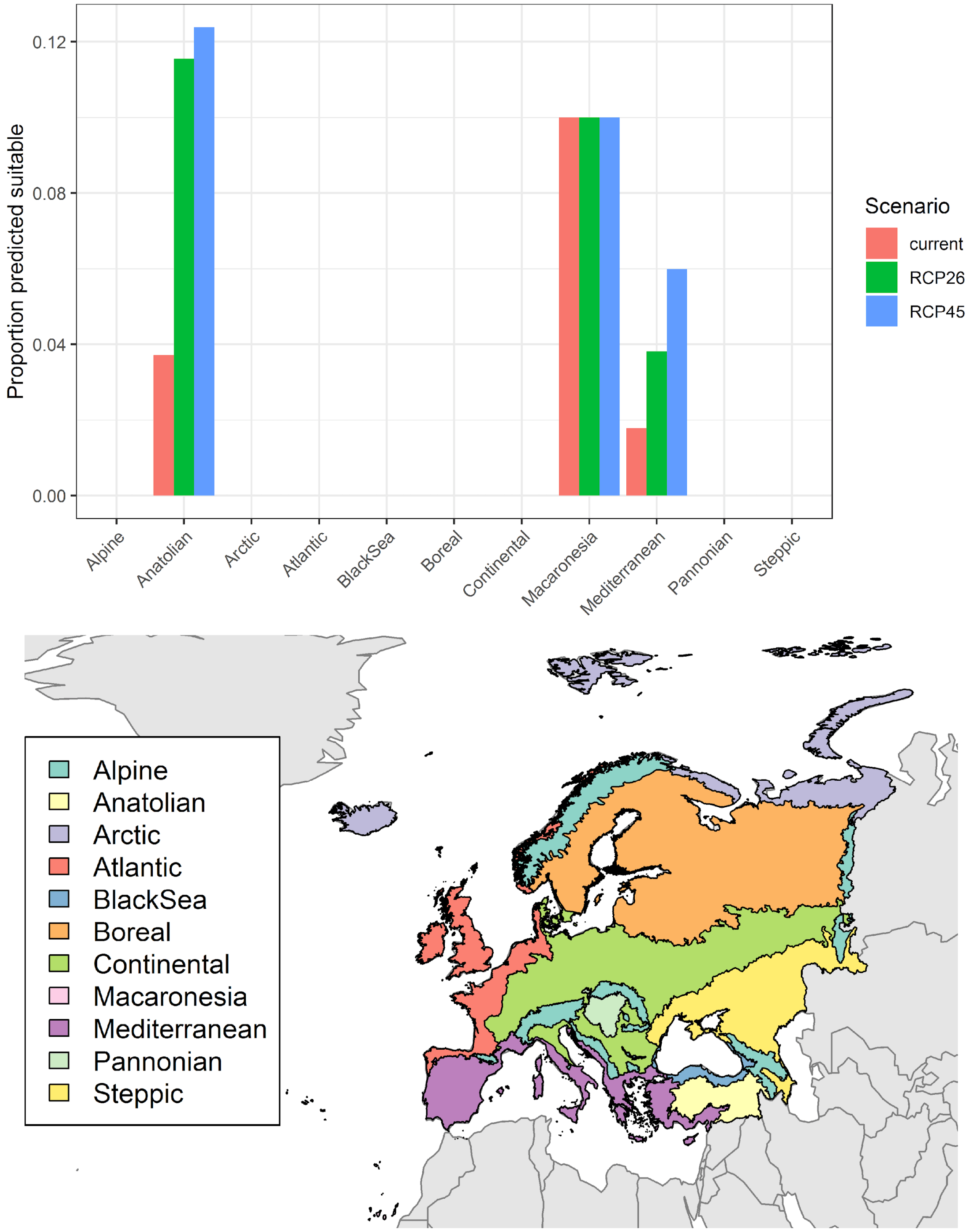
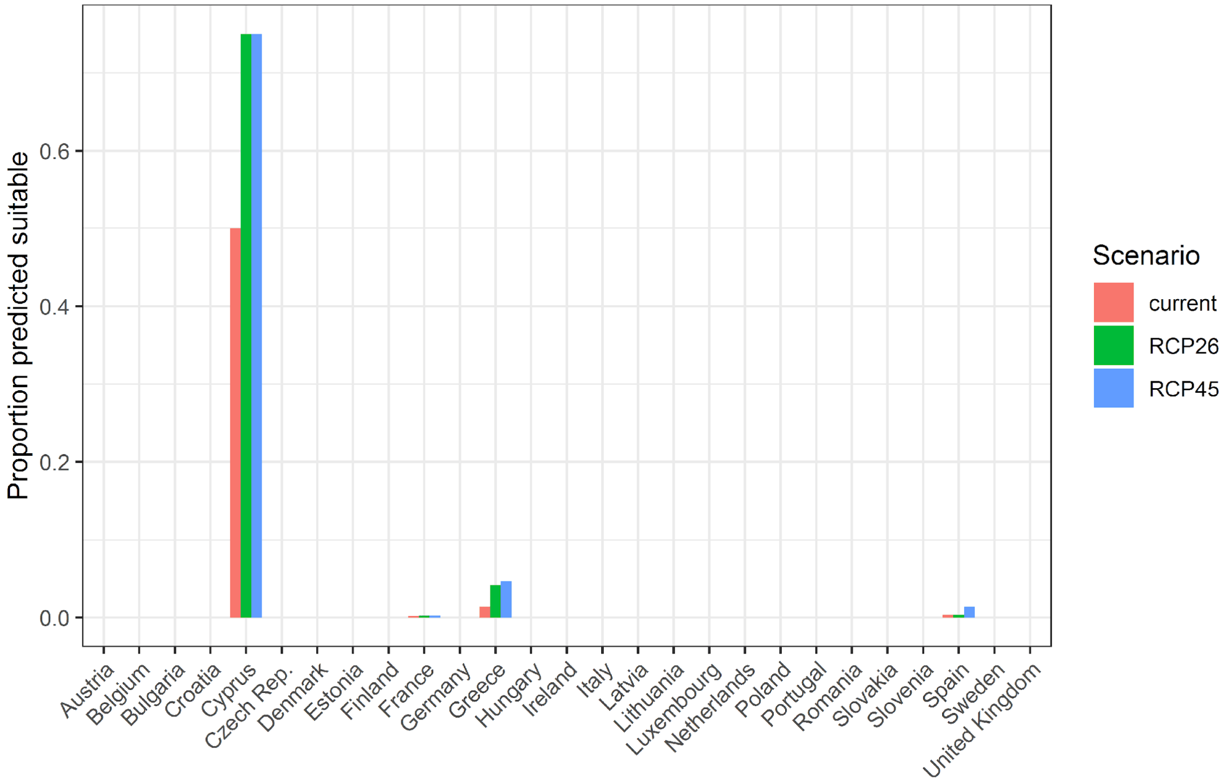


Figure 10. Variation in projected suitability for *Pycnonotus cafer* establishment among European Union countries. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.



Caveats to the modelling

To remove spatial recording biases, the selection of the background sample was weighted by the density of Aves records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	*Pycnonotus cafer*
Species (common name)	Red-vented Bulbul
Author(s)	Peter Robertson
Date Completed	04/04/2019
Reviewer	Tim Adriaens

Summary [1]

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Red-vented Bulbuls have been introduced through a variety of pathways, including the pet trade, deliberate introductions and via human assisted transport on ships. Control of these pathways and public awareness raising for this species and invasive alien species in general provides the most cost-effective method of ensuring this species does not establish in Europe.

This species is highly visible and not readily confused with any native European species. Given the popularity of birdwatching, early sightings are likely to arise through birdwatching reports and these provide a source of citizen science to help record this species.

Red-vented Bulbuls have been successfully controlled in a variety of eradication and management programmes in their introduced ranges. These programmes have typically involved a combination of shooting and the use of traps containing decoy birds. Details of suitable trap designs are publically available, traps containing decoy birds are particularly effective. Appropriate baits include fruits, particularly those with a red colouring. Mist nets may provide a useful method to catch small number of birds, for example as the initial decoys to use in traps.

A variety of toxins have been used to control this species, but there are no currently approved avian toxins suitable for use in the EU.

While a combination of trapping and shooting has been used to successfully control this species, no information on the costs associated with these programmes is available. Eradication will be most cost effective if started quickly after reports of an established population are first received.

This species can damage crops, particularly fruits but also plants themselves. A variety of chemical deterrents have proven to reduce foraging by this species under experimental conditions but have not been widely used in practice. Netting may also reduce crop damage in some circumstances.

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)[3]**	**Level of confidence[4]**
Methods to achieve prevention [5]			
Managing pathways	Red-vented Bulbuls have been introduced to new areas, probably through a variety of pathways, including the pet trade, deliberate introductions and via human assisted transport on ships (Turbott 1956). The adoption and enforcement of appropriate legislation and codes of best practice to reduce the risks posed by these pathways should reduce the probability of further introductions		
Effective surveillance and reporting	Red-vented Bulbuls are a highly visible species often found in association with human activity. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the species can become established.	Citizen-science species occurrence datasets are increasingly recognized as a valid tool for monitoring the occurrence and spread of invasive species across large spatial and temporal scales (Roy *et al.*, 2015). They are dependent on citizen-scientists who collect and upload data, typically from 'opportunistic sampling' with no underlying scientific survey design (Boakes *et al.*, 2010) which can limit the conclusions that can be drawn from these data (Isaac *et al.*, 2014). Most parts of north-west Europe have an extensive network of volunteer observers although this is less true of southern and especially eastern Europe (Boakes *et al.*, 2010).	

		Nevertheless the focus on native species, which is typical for monitoring activities carried out by bird-watchers may lead to disregard the presence of non-native species, and consequently to a delay in detecting a new presence of this non-native species. However, this naturalist community also provides an opportunity for developing an effective surveillance system. Unstructured citizen-science data do not reliably allow the estimation of species abundance or population trends (Kamp *et al.*, 2016), yet in an early-warning scenario it is likely sufficient to know where a species is establishing, and these data limitations are thus of a lesser concern.	
Raising awareness	Raising public awareness of the risks posed by invasive alien species in general and Red-vented Bulbuls in particular. This can include the production of targeted publicity and identification material.	A number of information sources and sheets are available on-line which can assist with the production of new material to raise public awareness. The Hawaii Invasive Species Council provide a handout describing the identification of the species and key information and links to other on-line sources. http://dlnr.hawaii.gov/hisc/info/invasive-species-profiles/red-vented-bulbul/	
Methods to achieve eradication[6]			
Trapping	A wide variety of designs are available for funnel entry or walk-in traps for birds. Traps must be monitored at least on a daily basis and any caught	Traps, in particular decoy traps, are an effective method to catch Red-vented Bulbuls and have been successfully used to support control programmes and eradications including New Caledonia, Tahiti, American Samoa and Fuerteventura (Thibault et al 2018, Cruz and Reynolds 2019). Thouzeau-Fonseca (2013) compared the different	High - Decoy traps are widely recognised as a successful method for capturing Red-vented Bulbuls and have been the used

	birds removed. Thouzeau-Fonseca (2013) describe a 'camembert' decoy trap that has proven effective for catching Red-vented Bulbuls. Red-vented Bulbuls have often been caught as part of Myna control programmes and traps for Mynas may also be suitable for this species. In relation to Mynas, Tindall (1996) and Tindemann (2005) describe wire netting cage-traps with walk-in funnel entrances that can also be used to contain a decoy bird. More recently Pham and van Son (2009), Saavedra (2010) and Feare (2017) describe a variety of trap designs and their practical use on Mynas. A wide range of myna traps based on these designs are commercially available and described on-line (search term 'myna trap'). Good trap sites are placed at sites with low levels of disturbance and open sites at least 3 m from vegetation that can harbour ground predators.	procedures of trapping in New Caledonia involved two types of cages, five types of fruit-baits (real fruit and fake fruit) and the presence or absence of a live bird as a decoy in the traps. In total, 59 bulbuls were captured. The most effective methods were the banana fruit-baits (46 % of the catches) and papaya (35 % of the catches), the presence of a decoy in the traps (76 % of the catches) and the cage-trap called "camembert" (68 % of the catches). This study compared two trap designs, a typical cage trap used for catching Mynas and a 'camembert' design involving a central 25cm wide decoy cage, surrounded by eight individual catching cages in a circular design, each with a treadle operated spring door. Photographs and trap designs are available in Thouzeau-Fonseca (2013) Thibault (2018) discusses the use of trapping alongside shooting as a control measure in New Caledonia. Trapping is a viable method for control as traps can be set safely in urban areas, villages, and forests. Previous research (Thouzeau-Fonseca 2013) has demonstrated the usefulness of the use of decoy birds associated with baits in trapping red-vented bulbuls and that reddish fruit baits can be efficient attractants (Thibault et al 2019). The trapping method should vary according to the context. In areas where intensive local trapping is needed (nearby sensitive area, extensive crop production area), aviary-type traps would probably be the most useful, allowing for multiple bird capture with minimal servicing of each trap. At locations where a light and movable trap is needed (invasion front, control in cities), smaller, more mobile traps	to support successful eradications

	Studies in New Caledonia have shown preferences for red coloured fruit baits for use to catch Red-vented Bulbuls (Thibault et al 2019). Captured birds can be humanely dispatched. Information on the humane dispatch of Mynas should also be applicable to Red-vented Bulbuls. The Pacific Island Initative (2013) have published best practice guidelines for euthanising Common Myna. Myna trap designs have also been produced which enclose the trap in a canvas sleeve which can then be flooded with CO^2 to humanely kill captured birds (Tidemann 2005, described in Pierce 2005). **Decoy-traps**: Any form of wire-netting trap that contains one or two decoy birds. The decoy birds are usually in a separate chamber and must be provided with shade, food and water	would be better. The use of a decoy bird can greatly enhance trapping success, but should be avoided when trapping birds at the edge of the distribution range to prevent any accidental release. Finally, trapping success will vary seasonally, with trapping success being highest when birds are searching for partners (spring) and when juveniles form flocks (late summer). Any control of a vertebrate is likely to attract some opposition, but live capture traps have been widely used in birds control programmes, for example control of the Myna, with the active cooperation of the public (Feare and Cruz 2009, Pham and van Son 2009). The use of decoy animals brings added complications, and potential welfare concerns. Decoys require food, water, perches, space to stretch and shelter when in the trap and care is needed to ensure their welfare. Traps are simple to manufacture and the skills should be available locally after adequate training. A range of effective designs have been published and similar traps for use on Mynas are commercially available online. The main costs of their use are manpower for setting and checking. Experience of trap placement is required for their effective use and this can take time to develop. The use of decoy birds and traps may be covered by local member state regulations. Local authorities should be asked for authorisation before their use.	

	(Sharp and Saunders 2004). The decoys serve to attract other birds towards the trap. Decoy traps are particularly effective when catching juvenile birds may also be effective for adults. Decoy birds can be used in most trap types if suitably modified. The traps must be frequently monitored.	Traps are likely to also catch other birds and mammals. Regular checking and the release of non-target species can reduce the risks posed. Traps are a relatively safe and benign method of capture. If used appropriately they do not pose particular health and safety, environmental, economic or social risks (although they can be mis-used to capture other protected species). The use of decoy traps combined with shooting is likely to be the most effective method to control or eradicate Red-vented Bulbuls, but needs to be used carefully to avoid trap-shyness. Other methods such as mist-netting and noose-trapping should be used in conjunction with traps to remove trap shy individuals and to catch the initial decoy birds.	
Shooting	Shooting can be an effective method to remove Red-vented Bulbuls. It is highly selective and, if used by experienced personnel, provides a humane method of despatch. It is labour intensive but has been used effectively to remove small numbers of birds during the early stages of establishment, and to remove trap shy individuals in other eradications of birds such as Mynas (Millet et al 2005, Feare et al 2017).	There was a successful Red-vented Bulbul eradication program implemented in New Zealand between 1952 and 1955 (Turbott 1956). This program allowed the early detection and shooting of bulbuls thanks to a reward associated with a call for information and led to an announcement of eradication in 1955 (Watling 1978). This management strategy remains in place in New Zealand and it helped prevent establishment following two more recent introduction events (September 2006 and February 2013) (Thibault et al 2018). Shooting has also formed part of the control programme on Tahiti (Cruz and Reynolds 2019). In New Caledonia, Thibault (2018) advocates shooting by local hunters through a collaborative and participative management approach. However, he notes that a major	High -Shooting has been used to remove small populations of Red-vented Bulbuls.

		constraint to this method is the current distribution of the Red-vented Bulbul in inhabited areas, where shooting is forbidden. This explains why current control actions are conducted in rural areas at the edge of the distribution range The use of different firearms is heavily regulated and the details vary between member states. These are likely to restrict the nature of the weapon, the requirements for the operator and the times and locations where they may be used. Local authorities must be consulted before their use. The use of firearms brings risks to health and safety which need to be managed. Its use in public places is likely to bring opposition and raise particular concerns. The use of lead projectiles has been restricted in some areas due to environmental concerns, although non-toxic alternatives are available.	
Mist-nets	Mist nets are fine polyester or nylon nets which are suspended between two upright poles, requiring continual monitoring and expert handling of caught birds (Sharp and Saunders, 2004).	Mist nets are commonly used for the live-capture of birds for ringing. Appropriate equipment and experienced personnel should be locally available. Experience and training is required to safely extract birds from the nets and a number of EU member states run approval and licensing schemes for their use. The use of mist nets may be covered by local member state regulations. Local authorities should be consulted before their use. Mist nets are unselective and are likely to catch birds of a range of species. However, trained personnel should be able to extract and release any non-target species caught. Mist	Moderate – mist nets are widely used for the capture of other bird species and may be of use against Red-vented Bulbuls

		nets need to be used under constant supervision to quickly remove captured birds, reducing their cost-effectiveness compared to other methods. Mist nets are widely used for bird ringing, their use poses few risks to health and safety, the environment, economy or society. Mist nets have not been reported as a method used for the control of Red-vented Bulbuls, but may be useful in some circumstances, for example the capture of initial decoy birds for use in traps.	
Use of toxins	Spurr and Eason (1999) review the available avicides worldwide. The two most widely used compounds are Starlicide (©DCR-1339, 3-chloro-p-toluidine hydrochloride) (Ramey et al., 1994, ACVM, 2002), and the stupifactant Alphachloralose ((R)-1,2-0-(2,2,2-trichloroethylidene)-α-D-glucofuranose) (Nelson, 1994; Thearle, 1969). Cruz and Reynolds (2019) report that Starlicide has been an effective toxicant used against Red-vented Bulbuls in American Samoa. However, no products are currently approved for use in	The lack of approved compounds effectively prevents the use of toxicants for bird control in the EU. Consequently these methods are not considered in great detail in this review. Starlicide is not currently approved for use in the EU. In the past, Alphachloralose has been approved as an avicide and was used in a number of EU states (Ridpath et al., 1961, Spurr and Eason, 1999). However, it is not currently registered for use as an avicide in the EU. It is currently approved for use against rodents (EU Directive 98/8/EC 2008). Although toxins can be an effective method of bird control, there are significant concerns regarding their non-target effects and public acceptability. In New Caledonia, toxicants have not been used as part of their Red-vented Bulbul control as their use is considered non-selective and to pose an unacceptable risk to other non-target species (Thibault	High. There is an existing literature on the use and effectiveness of these compounds and examples of their use on Red-vented Bulbuls in areas where their use has been approved.

	the EU.	2018).	
Methods to achieve management [7]	All of the methods described to support eradication can also be used to manage existing Red-vented Bulbul populations.	See above	See above
	Protecting fruit and plants from damage. To reduce damage to commercial fruit crops, it may be possible to apply chemical feeding deterrents. Avery (2003) reviews the methods and use of chemical bird deterrents. A cage test conducted in Hawaii on bird repellant showed that Ziram, Methiocarb and Methyl anthranilate reduced the consumption of treated papaya mash by Red-vented Bulbuls (Cummings et al. 1994). In an open-field test, the same authors showed that Methiocarb significantly reduced damage on orchids. Netting of valuable crops can also be used. Thibault et al (2019) describe netting to protect tomato plants from	Netting and the use of chemical deterrents have not been widely used to protect fruits from damage by this species, most evidence arises from scientific studies rather than their practical application. The economics of their use remain undetermined. The use of netting brings a range of issues related to practicality as nets need regular maintenance and are susceptible to damage. Chemical deterrents require licensing before use. The use of nets is likely to be acceptable to the general public, the use of chemical deterrents may raise concern if the fruits are destined for human consumption	Medium. These methods have been used to reduce damage by other bird species, but there is only limited information related to Red-vented Bulbuls

	damage by this species.		

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.

- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; this is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: ***Fallopia baldschuanica*** **(Regel) Holub**

Author(s) of the assessment:

Rob Tanner, EPPO, Paris, France
Richard Shaw, CABI, Egham, GB
Johan van Valkenburg, Wageningen, Netherlands

Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: *Giuseppe Brundu,* University of Sassari, Department of Agriculture, Italy
Peer review 2: *Etienne Branquart,* Service Public de Wallonie, Belgium

Date of completion: 17th September, 2019

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:
There are a few controversial interpretations on the taxonomy and nomenclature for *Fallopia aubertii* (L.Henry) Holub, Folia Geobot. Phytotax. 6: 176 (1971) and *Fallopia baldschuanica* (Regel) Holub, Folia Geobot. Phytotax. 6: 176 (1971).

Although these two entities are in some cases considered and traded as two distinct species, for the purposes of the present risk assessment they are **considered as synonyms and they are included in a single unit**, i.e. *Fallopia baldschuanica* s.l. *(incl. Fallopia aubertii).* This is in accordance, e.g. with Flora Europaea (Tutin et al., 1993), with the Flora of China (2019) and with the Flora of North America (2019).

In addition, the New Flora of the British Isles, Stace (2019) details that *F. aubertii* “perhaps differs in its smaller achenes and flowers, pinker perianth and more undulate leaf-margins: it appears to be rare in cultivation [in Great Britain] but is very doubtfully specifically distinct (perhaps better as a ssp.), and other differences claimed are not constant”.

Similarly, the European Garden Flora (Stuart Max Walters, Cambridge University Press, 1984) considers the two taxa as distinct species (under the names, *Polygonum aubertii* L.Henry and *Polygonum baldschuanicum* Regel) but also remarks that they are very similar, and the differences between them are not apparently always consistent so that they should be probably regarded as subspecies of the same species.

Taxonomy:
Kingdom: Plantae;
Phylum: Magnoliophyta;
Class: Angiospermae;
Order: Caryophyllales;
Family: Polygonaceae
Genus: *Fallopia*

Valid name: *Fallopia baldschuanica* (Regel) Holub, Folia Geobot. Phytotax. 6: 176 (1971).

Basyonym: *Polygonum baldschuanicum* Regel, Trudy Imp. S.-Peterburgsk. Bot. Sada, 8 : 684 (1883)

Synonyms (from Plant List, 2019, Stace, 2019):

Polygonum aubertii L.Henry, Rev. Hort. 79: 82 (1907)
Fagopyrum baldschuanicum (Regel) Gross, Bull. Acad. Int. Géogr. Bot. 23: 21 (1913)
Bilderdykia aubertii (L.Henry) Moldenke, Revista Sudamer. Bot. 6: 29 (1939)
Reynoutria aubertii (L.Henry) Moldenke, Bull. Torrey Bot. Club 68: 675 (1941)
Reynoutria baldschuanica (Regel) Moldenke, Bull. Torrey Bot. Club 68: 675 (1941)
Tiniaria aubertii (L.Henry) Hedberg ex Janch., Phyton (Horn) 2: 76 (1950)
Tiniaria baldschuanica Hedberg ex Janch., Phyton (Horn) 2: 75 (1950)
Bilderdykia baldschuanica (Regel) D.A.Webb, Feddes Repert. Spec. Nov. Regni Veg. 68: 188 (1963)
Fallopia aubertii (L.Henry) Holub, Folia Geobot. Phytotax. 6: 176 (1971)

English common names: Bukhara fleece-flower, Chinese fleecevine, mile-a-minute vine, Russian vine, silver lace vine

Other languages: grmolika heljda (Croatian), sølvregn (Danish), bruidssluier (Dutch), muuritatar (Finnish), renouée de Boukhara, renouée du Turkestan (French), bucharischer Windenknöterich (German), nashiyuki-kazura, ナツユキカズラ (Japanese), klatreslirekne (Norwegian), rdest bucharski, rdestówka bucharska (Polish), cascata, cordão-prateado (Portuguese), горец бальджуанский, гречиха бальджуанская, гречишка бальджуанская (Russian), grmasti slakovec (Slovene), polígono enredadera, polígono ruso, viña del Tíbet, viña rusa (Spanish), bokharabinda (Swedish).

Description of the species:

The online flora of North America[2] describes the species as following:

- Vine, perennial, not rhizomatous, 3-10 m.
- Stems climbing, branched from near base, woody, glabrous.
- Leaves: ocrea usually deciduous, hyaline or brownish, cylindric, 3-8 mm, margins truncate to oblique, face glabrous throughout; petiole 1-4 cm, glabrous or scabrid; blade narrowly ovate to ovate-oblong, 3-10 × 1-5 cm, base subcordate or cordate to sagittate, margins entire or wavy, glabrous or scabrid, apex obtuse to acuminate, abaxial face glabrous or scabrid along midvein, rarely minutely dotted, not glaucous, adaxial face glabrous. Inflorescences axillary and terminal, spreading or drooping, paniclelike, 3-15 cm, axes glabrous or papillate to scabrid in lines; peduncle 1-3 cm, glabrous or scabrid.
- Pedicels ascending or spreading, articulated proximal to middle, 1.5-4 mm, glabrous or scabrid.
- Flowers bisexual, 3-6 per ocreate fascicle; perianth accrescent in fruit, greenish white with white wings or mostly pink, sometimes bright pink in fruit, 5-8 mm including stipelike base, glabrous; tepals elliptic, apex obtuse to rounded, outer 3 winged; stamens 6-8; filaments flattened proximally, pubescent proximally; styles connate basally; stigmas peltate.
- Achenes included, dark brown to black, 2-4 × 1.8-2.2 mm, shiny, smooth; fruiting perianth glabrous, wings flat to undulate, 2-4 mm wide at maturity, decurrent on stipelike base nearly to articulation, margins entire. $2n = 20$ (Korea).

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[2] http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242100054

Hybrid × *Reyllopia conollyana*

A rare hybrid has been recorded within the EU between *Fallopia baldschuanica* and *Reynoutria japonica*, i.e. × *Reyllopia conollyana* (J.P.Bailey) Galasso (*Fallopia* x *conollyana* J.P.Bailey, *Reynoutria* x *conollyana* J.P.Bailey. Bailey & Spencer (2003) details that the hybrid is known to exist in the UK since 1983 and as an established species since 1986. There are also a number of records of the hybrid in continental Europe (Bailey, 2001).

The Manual of the Alien Plants of Belgium (2019) highlights that the hybrid has been recorded from two localities in Belgium. The first observed on a talus slope in Izegem in 2016 and later in the same year also alongside a railway track near Ghent (Hoste *et al*. 2017). These records refer to established plants. In addition, this hybrid has also been recorded as open pollinated seed from *F. japonica*. It is probably not rare as such but, for some reason, rarely establishes itself in the wild.

Description of the hybrid × *Reyllopia conollyana*:

Herbaceous perennial, younger plants weakly rhizomatous, but stout woody rhizomes in long established plants; stems erect but bowing over, hollow, up to 2 m long, slender with red blotches, less than 1cm diameter, becoming woody at the base. Leaves are glabrous with slender petioles 0.5 – 1.5 cm; lamina up to 13 x 6·5 cm, triangular ovate, acuminate to acuminate cuspidate at apex, subcordate to truncate at base; inflorescence axillary and terminal panicles. Flowers are superficially similar to, but larger (up to 3 mm diameter) and more conspicuous than those of *F. japonica*, the 3 keeled petals much more conspicuously winged than those of F. japonica; style trifid with 3 club shaped stigmata with short fimbriae (resembling more closely the stigmas of the *F. japonica* parent). Flowering occurs from September to early October. In spite of the woody lignified stem with buds, the plant is herbaceous, overground growth dying back to ground level or near ground level in winter, to be replaced by new canes in the spring. 2n=54.

There is no evidence that × *Reyllopia conollyana* occurs in the RA area as a species in horticulture. Additionally, as the hybrid is extremely rare in the natural environment, it is not considered further in this risk assessment. There may also be the potential for hybridization of *Fallopia baldschuanica* with other *Fallopia* species, but this will need further assessment.

The hybrid may be confused with *F. baldschuanica* as the form of the former shows reasonable levels of morphological diversity. However, some features to distinguish the two apart include:

- *F. baldschuanica* would produce significant amounts of flowers which will look like a mat of white during summer months. × *Reyllopia conollyana* will flower much less.
- *F. baldschuanica* growth is typical of numerous vines smothering vegetation or manmade structures, whereas × *Reyllopia conollyana* will often grow with a small number of erect vines.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);

- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

Fallopia dumetorum is a native species to the EU and it could be misidentified as *F. baldschuanica.* There is no evidence that the species is available in horticulture within the EU. *Fallopia baldschuanica* may also be confused with *Anredera cordifolia,* a South American native, which is available in horticulture in the EU.

Particularly in relation to Great Britain, GB NNSS (2011) detail: “other perennial climbers with triangular to heart-shaped leaves are Bindweeds. Two native species are Hedge bindweed (*Calystegia sepium*) and field bindweed (*Convolvulus arvensis*). Both have distinctive trumpet-shaped flowers white, pink or pink with white stripes. Black bindweed (*Fallopia convolvulus*) is native throughout Europe and has similar flowers to Russian-vine but its lower stems are not woody. Large bindweed (*Calystegia silvatica*) is native to the EU (e.g. Spain, France, Italy etc.) and is very similar to Hedge bindweed and is only distinguished by leafy structures at the bases of the flowers”.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: There are no known risk assessments for *F. baldschuanica,* either for the EU or other regions of the world.

To note:

F. baldschuanica was however considered in different non-native species horizon scanning studies within the European Union, with contrasting results and low to medium confidence levels (see table).

Table 1 - Priority of *F. baldschuanica* for risk assessment evaluated through different horizon-scanning studies performed in Europe.

Study	**Area under assessment**	**Priority for risk assessment**	**Confidence level**
Brunel et al (2010)	EPPO region	High priority	Low
Thomas (2010)	Great Britain	High priority (critical)	-
Pergl et al (2016)	Czech Republic	Medium priority (black list species whose spread depends on human activities)	-

Tanner et al (2017)	European Union	Medium priority (observation list)	Medium
Carboneras et al (2018)	European Union	Low priority (minor impact)	Medium
Invasive species Ireland (2013)	Ireland	Medium priority (amber list)	Low

Brunel *et al*. (2010) prioritized *F. baldschuanica* for the EPPO region using the EPPO prioritization process for invasive alien plants and the results indicated that the species had a medium spread potential with a high impact on the environment. Brunel *et al.* (2010) notes that there is no information on potential impact on agriculture and forestry. Brunel *et al.* (2010) citing Sanz Elorza *et al.* (2004) states "this vine grows over shrubs and trees, and along riparian forests [in Spain]. Brunel *et al.* (2010) goes on to highlight that the uncertainty for the prioritization assessment is considered high as there is very little data on the species.

The species is ranked as critical and recommended as a priority for more detailed risk assessment in the horizon-scanning study in Great-Britain, e.g. because of good climatic suitability, spread within the country, vegetative reproduction, climbing behavior and formation of dense thickets (Thomas 2010).

In Czech Republic, it is ranked as a medium priority for risk assessment due to limited intrinsic spread capacity and moderate environmental impact (Pergl et al . 2016).

Within the initial prioritization of 37 invasive alien plants for the EU (LIFE Project: Mitigating the threat of invasive alien plants in the EU through pest risk analysis to support the EU Regulation 1143/2014), *F. baldschuanica* was prioritized. Spread was assessed as medium and impact was evaluated as medium on native plant species (biodiversity) and medium impact on ecosystem functions and services. As a result, the species was not taken through to the second stage to assess its priority for a risk assessment (Tanner et al 2017).

The species was not ranked as a priority species for risk assessment in the horizon scanning study of Carboneras et al. (2018).

Invasive Species Ireland (2013) have evaluated the species and assigned a score of 14 for the species, placing it on the Amber List (uncertain risk). The Amber list (uncertain risk) includes species rated as medium risk due to the score of the overall assessment however, their impact on conservation goals remains uncertain due to lack of data showing impact (or lack of impact).

Fallopia baldschuanica was added to the EPPO Alert List in 2007 and transferred to the EPPO List of Invasive Alien Plants in 2012[3].

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is

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[3] https://www.eppo.int/ACTIVITIES/invasive_alien_plants/iap_lists

naturally occurring

- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: *Fallopia baldschuanica* is native to Asia. EPPO (2012) list in particular the following countries: Afghanistan, China (Tibet), Pakistan (Waziristan in the north-east), Russia (south, Siberia), Tajikistan.

The Flora of China (2019) detail that *Fallopia aubertii* (*Fallopia baldschuanica)* occurs in the following Provinces: Gansu, Guizhou, Henan, Hubei, Hunan, Nei Mongol, Ningxia, Qinghai, Shaanxi, Shanxi, Sichuan, Xizang, Yunnan.

There is no specific knowledge on climatic zones and habitats where the species occurs in its native area.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: The Global Biodiversity Information Facility (GBIF, 2019) record the locations of the species in New Zealand, North America (United States, Canada and Mexico), Central America (Costa Rica). Q Bank (2019) also record the species present in the United States (including Alaska).

The Flora of North America (2019) detail that *Fallopia baldschuanica* is present in California, Colorado, Maryland, Massachusetts, Michigan, New Jersey, New Mexico, New York, Pennsylvania, Utah, Virginia, and Washington. The Flora of America (2019) also detail the species has been introduced in Central America (Costa Rica) and Europe.

The GloNAF Database (2019) (Global Naturalised Alien Flora) details that *F. baldschuanica* is naturalised in Liechtenstein.

Fallopia baldschuanica has been recorded as a casual alien in urban situation in Bosnia and Herzegovina (Amslo and Boškailo, 2018; Maslo, 2014, Maslo and Abadžić, 2015; Maslo and Boškailo, 2018).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a): Alpine, Atlantic, Continental, Mediterranean,

Response (6b): Atlantic, Continental, Mediterranean.

See answer to question A8 for the sources of information.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Under the current climatic conditions, it is predicted that *F. baldschuanica* will be able to (further) establish within the biogeographical regions where the species is already established: the Atlantic, Continental and Mediterranean biogeographical regions. In addition, further establishment could occur in all biogeographical regions where the species is planted (see Annex VII, Figure 5).

This would additionally include: Boreal, Black Sea, Alpine, Pannonian and Steppic biogeographical regions.

Response (7b): Under a climate change scenario of (RCP 4.5, over the next 30/50 years), the Atlantic and Continental biogeographical regions are envisaged to remain suitable for the establishment of the species. Areas of the Mediterranean may become more limited for the establishment of the species due to increased temperature and more increased drought periods. With climate change, areas of the Boreal, Black Sea and Alpine biogeographical regions may become suitable for the establishment of the species (see Annex VII, Figure 8).

Note: it is the opinion of the authors of the RA that the SDM may over represent the potential establishment of the species in the natural environment within the RA area as the data taken from GBIF to perform the models would also include localities where the species has been planted and therefore this is not representative of the natural occurrence and establishment of the species in the natural environment.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom.

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):
Austria (Fischer *et al*., 2008; Stöhr *et al*., 2012, Essl and Stöhr, 2006),
Belgium (Verloove 2002, 2006),
Bulgaria (DAISIE), Croatia (DAISIE),
Czech Republic (Pyšek *et al*., 2012),
Denmark (NOBANIS),
France (GT IBMA, 2016),
Germany (Buttler et al., 2018; floraweb, 2019),
Great Britain (GB NNSS, 2011, Stace, 2019),
Greece (Arianoutsou *et al*., 2010),
Hungary (DAISIE),
Ireland (https://species.biodiversityireland.ie/profile.php?taxonId=29164#Status),
Italy (Portal of the flora of Italy, 2019; Galasso et al., 2018; Ballelli *et al*., 2009; Selvaggi *et al*., 2009),
Luxembourg (pers comm. E. Branquart, 2019),
Netherlands (https://www.verspreidingsatlas.nl/2486#) Portugal (De Almeida and Freitas, 2006),
Romania (DAISIE),
Slovakia (DAISIE),
Slovenia (Krajsek and Jogan, 2011; Veenvliet *et al*., 2017),
Spain (Vilà *et al*., 2012).

Response (8b): Austria (Essl, 2005), Belgium* (Verloove 2002, 2006), Bulgaria* (DAISIE), Czech Republic* (Pyšek *et al.*, 2012), France* (GT IBMA, 2016), Germany* (Buttler et al., 2018), Great Britain* (UK NNSS 2011, Stace, 2019), Ireland* (https://species.biodiversityireland.ie/profile.php?taxonId=29164#Status), Italy* (Portal of the flora of Italy, 2019; Galasso et al., 2018; Ballelli *et al.*, 2009; Selvaggi *et al.*, 2009), Portugal* (De Almeida and Freitas, 2006), Romania, Slovenia (Veenvliet *et al.*, 2017), Spain* (Vilà *et al.*, 2012).

* The GloNAF (Global Naturalised Alien Flora) Database details that in these countries the species is naturalized.

Fallopia baldschuanica was very likely introduced in Europe by Ward (using the wardian case) in 1890 from Russia (Bokhara) as reported by Christopher Thacker (The History of Gardens, University of California Press, 22 Oct. 1985 - 288 pp).

Additional Member State information (where available):

Austria: *Fallopia baldschuanica* has been observed growing in and around urban habitats and is considered to be locally naturalized (Stöhr *et al.*, 2012, Essl and Stöhr, 2006). Essl (2005) report the species growing close to reservoir southwest of Kleedorf.

Belgium: the species was first recorded outside cultivation in 1942 in the sea dunes in Duinbergen (Knokke).

Denmark: NOBANIS (2019) detail that the first report of the species was in 1977 (see also Hanse (1978), Hartvig *et al* (1988) and Madsen *et al* 1991)).

France: the species was first observed in the natural environment in the department of Puy-de-Dôme in 1904. *Fallopia baldschuanica* (named as *Fallopia aubertii*) has been included in list four (priority lists developed by Conservatoire Botanique de Franche-Comté" (a Department in Eastern France). The 49 species on this list are not yet considered invasive in Franche-Comté, but have shown invasive behaviour in other areas of France, or in other countries. Observations are reported from semi-natural habitats like dune ecosystems and riparian habitats (Fried 2014).

Germany: Buttler et al. (2018) highlight that *F. baldschuanica* is established in Germany but is casual in some of the Federal States.

Great Britain: GB NNSS (2011) detail that the species was introduced into the UK towards the end of the 19th century and was first recorded in the wild in 1936. Stace (2019) highlight that the species is scattered over most of the British Isles but scarce in Ireland and Scotland. Stace (2019) detail that the species is commonly cultivated and a 'persistent throw-out or relic in waste scrubby places or hedges…'.

Greece: Arianoutsou *et al.* (2010) detail that the species has been introduced into Greece, but it is currently not established in the wild.

Ireland: The National Biodiversity Centre Ireland (https://species.biodiversityireland.ie/profile.php?taxonId=29164#Status) detail that *F. baldschuanica* was first reported in 1970 and highlight that the species is regarded as established. In Ireland, the species is reported as familiar garden plant and occasionally encountered in other situations but nearly always planted.

In Italy, the species is present (from casual to invasive) in all the Italian administrative regions, with the exception of the region Calabria, in the south (Portal to the flora of Italy, 2019). Podda *et al* (2011) detail *F. baldschuanica* as naturalised in Sardinia. The species is recorded as being naturalised in Sicily though Bazan and Castellano (2007) highlight that the species has been observed in large local populations but shows little invasiveness.

Netherlands: Locally naturalized as a garden escape, or result of dump of garden waste (Mennema & Holverda 1983; https://www.verspreidingsatlas.nl/2486#)

Portugal: De Almeida and Freitas (2006) report that the species was first reported as naturalised in Portugal in 1965.

Slovenia: the species was first found in the natural environment in 1980 (Project LIFE ARTEMIS see https://www.tujerodne-vrste.info/en/project-life-artemis/). The ARTEMIS projects website details habitats as: ruderal sites, forest edges, riverine forests, mostly in the vicinity of inhabited areas. The species is on the Alert List in Slovenia.

Spain: The species is a regulated invasive alien plant in Spain as of 2013 (https://www.boe.es/buscar/act.php?id=BOE-A-2013-8565). Their possession, transport, movement, trade of live or dead specimens or propagules are also prohibited within Spain, as well as their export. Authorisations may nevertheless be granted for research or health reasons. Brunel *et al.* (2010) detail that *Fallopia baldschuanica* was first introduced into Spain in 1889 and citing Sanz Elorza *et al.* (2004) states “this vine grows over shrubs and trees, and along riparian forests [in Spain]”.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): At present *F. baldschuanica* is established in the wild in Austria, Belgium, Bulgaria, Czech Republic, France, Germany, Great Britain, Ireland, Italy, Portugal, Romania, Slovenia, Spain. It is envisaged that further establishment would be seen in these countries and other countries where the biogeographical regions are the same. Additional countries to the aforementioned would be the Netherlands, Denmark, Luxembourg, Croatia, Slovakia, Romania, Poland, Greece.

Response (9b): Under a climate change scenario of (RCP 4.5, over the next 30/50 years), countries in northern Europe may become suitable for the establishment of *F. baldschuanica* due to the increased temperature and the length of the growing season. These countries would include Lithuania, Latvia, Estonia, Finland and Sweden. All remaining countries listed in 9a will remain suitable under RCP. 4.5.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: The information on the invasiveness of *F. baldschuanica* in areas outside the RA area is very limited. The authors of the RA are not aware of any scientific study evaluating the impacts of the species on biodiversity.

However, the species has been highlighted as having invasive tendencies outside of the RA area on various online sources. In Washington State (Washington State Noxious Weed Control Board, 2019), *F. aubertii* (*F. baldschuanica*) is on the monitor List, where the purpose of the monitor list is to gather more information on suspect weeds, as well as monitor for occurrence or spread. There is no legal or regulatory aspect to this list. Plants on the monitor list are not listed noxious weeds in Washington. King County (2019) detail that *F. baldschuanica* has shown to escape cultivation and grow rampantly up and over other vegetation including even very tall trees. King County (2019) state: because it grows over other plants, it can suppress their growth and weigh them down. It is highly branched and difficult to remove from other plants without injuring them. It grows over low-lying vegetation as well as climbing high into trees and growing over the tops of tall plants, even other invasive plants such as blackberry and knotweed.

The University of Connecticut (2019) highlight that the species has demonstrated an invasive tendency in Connecticut meaning that it may escape from cultivation and naturalize in minimally managed areas.

Martine *et al* (2009) highlight that *F. baldschuanica* has been identified as a potential problem in a number of US States and escaped populations have been recorded in at least 11 States. They highlight that the species has the potential to smother native vegetation and unmanaged populations can easily take over adjacent plantings.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Atlantic, Continental, Mediterranean,

The species has shown invasiveness (i.e. fast spread and smothering of native vegetation) in the Atlantic, continental and Mediterranean where the areas have enough water availability for the plant to develop.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response: Czech Republic (Pyšek *et al*., 2012; Pergl et al 2016), Ireland (Invasive Species Ireland, 2019), Italy (Portal of the flora of Italy, 2019; Galasso et al., 2018; Ballelli *et al*., 2009; Selvaggi *et al*., 2009), Portugal (De Almeida and Freitas, 2006), Slovenia (Veenvliet *et al*., 2017), Spain (Vilà *et al*., 2012), United Kingdom (Thomas et al 2010, Stace, 2019).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response: *Fallopia baldschuanica* is a commonly grown garden ornamental species within the RA area. The species is widely traded within the RA area and often sold for its ability to quickly colonise hedge gaps or grow up bare garden walls and as a ground cover along roads and railways. The Royal Horticultural Plant Society (Plant Finder: www.rhs.org.uk) highlights 30 suppliers of the species throughout the UK alone. The species is on sale on eBay (7 items found in July 2019) from the UK and USA.

The species is also grown as an amenity plant, to cover buildings and other urban developed sites.

The therapeutic potential of the species has received some consideration though clear benefit of the species have yet to be identified (Olaru *et al* 2013). The species anticancer potential has been assessed and there is some evidence that it may represent a good source of plant extracts with anticancer properties (Olaru *et al*., 2015).

Dénes *et al*. (2012) detail that *F. baldschuanica* leaves have been used by ethnic groups living in the Carpathian Basin. The leaves can be used as a vegetable “with a meaty stuffing filled into them: leaves were used as a children’s snack”.

Ber (1957) used cut green material in a study for the use of *F. baldschuanica* as a fodder crop noting that when compared with the nutritive value of other fodder plants, protein remains fairly constant between May and October and recommends that the plant be grown for use as fodder.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: "No information has been found."
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as "corridor" or "unaided)".
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[4] and the provided key to pathways[5].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9

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[4] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[5] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Pathway name: Horticulture

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	**CONFIDENCE**	high

Response: Introduction via this pathway is deliberate, and planting of the species would be the end result of the movement of the species. *F. baldschuanica* is a commonly grown garden ornamental species within the RA area. The species is widely traded within the RA area and often sold for its ability to quickly colonise hedge gaps or grow up bare garden walls. The Royal Horticultural Plant Society (2019) (Plant Finder: https://apps.rhs.org.uk/horticulturaldatabase/) highlights 30 supplies of the species throughout the UK alone. The species is on sale on eBay (7 items found in July 2019) from the UK and USA. It is likely that the species is sold throughout the RA area as an ornamental species.

Note: There is a lack of information of the viability of seed and the capacity of the species to grow from seed. This information is sparse from both the introduced and native range. Of the few references to viability to seed, Tiebré *et al*. (2007) did not observe any seed germination during experimentation from seed sources from Belgium. However, GB NNSS (2011) state that the species has the capacity to produce viable seed, though seedlings are relatively infrequent.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: *Fallopia baldschuanica* is available through the horticultural trade both within the RA area and outside. The species is available via internet suppliers (e.g. ebay.com from the US and the UK) but it remains unclear if the species can be sent to buyers within the EU from the USA.

The majority of plant stock is likely to be produced within the RA area and thus volumes entering the RA area may potentially be low, however there is no information on the frequency of passage through this pathway into the RA area.

Whole plants and potentially cuttings could enter the RA area via plants for planting. Seeds are also available from amazon.com (see https://www.amazon.com/SILVER-LACE-Polygonum-Aubertii-SEEDS/dp/B011GX0M6Q) and ebay.com (see https://www.ebay.ie/itm/SILVER-LACE-VINE-Polygonum-Aubertii-10-SEEDS-FREE-S-H-/223453049734?hash=item3406d6f386).

The authors of this RA consider that a low propagule pressure could result in the introduction of the species into the RA area. The species can readily grow from cuttings and fragments of the stem. Propagule pressure from seed is unknown, there is no information available for this aspect.

As entry via this pathway is deliberate, and planting of the species would be the end result of the movement of the species low numbers of propagules could result in the introduction of the species.

A likely score has been given with a moderate confidence, where the latter reflects the uncertainty on the volumes of stock material introduced into the RA area from outside.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: The pathway ‘Horticulture’ is the deliberate movement of plant material into the risk assessment area and as such plant material would be maintained and moved to ensure survival. No management practices would be carried out along this pathway.

Live plants rooted in growing media are the most likely form of the species to be introduced in the RA area. Cuttings of the plant may also be introduced in the RA area. The species is known to be a hardy species (RHS refer to the species as fully hardy: https://www.rhsplants.co.uk/plants/_/fallopia-baldschuanica/classid.1701/).

It is unlikely that the species will reproduce along the pathway. Both cuttings and whole plants could be moved along this pathway. As mentioned above, seeds are also traded via ecommerce.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: No management practices would be carried out specifically on the species along this pathway. The species itself is the commodity.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: It is unlikely that the organism will enter the risk assessment area undetected via the pathway ‘Horticulture (escape from confinement)’ as this is the deliberate movement of plant material into the risk assessment area.

Although some species can be introduced in the RA area as contaminants of plants for planting, it is unlikely that live plants of *F. baldschuanica* will be introduced in the region in this form as the smothering behaviour of the species would lead to it being detected and removed from potted plants.

It should be noted that the achenes are small 4-5 mm (Stace, 2019) and thus they could be introduced into the RA area as a contaminant of plants for planting, however the authors of this RA consider this is not realistic.

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: The species is traded and is available via the internet trade from both within and outside of the RA area. A likely estimate of the overall likelihood of introduction has been given as there is the potential that large amount of the species is not traded from outside into the RA area. What is more likely is that the stock of plant material is sourced from within the RA area.

Pathway name: Ornamental purposes other than horticulture

Qu. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	**CONFIDENCE**	high

Response: Introduction via this pathway is deliberate, and planting of the species would be the end result of the movement of the species. *F. baldschuanica* can be planted in urban landscapes to ‘green’ areas. Fences, walls and buildings, along with road verges are all urban habitats where the species may be planted to hide man-made structures. These plantings may also be used to cool buildings where an article in Gardeners World highlight the use of the species to cool walls in urban habitats (Boeri, 2018).

In addition, the occurrence of the species around areas like Heathrow Airport (GB), suggest the species has been deliberately planted for its fast growth and ability to form mats. However, there is no information available on deliberate planting of the species for these purposes other than personal observations.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: The majority of plant stock is likely to be produced within the RA area and thus volumes entering the RA area may potentially be low, however there is no information on the frequency of passage through this pathway into the RA area.

See Qu. 1.3a for additional information.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: This pathway involves the deliberate movement of plant material into the risk assessment area and as such plant material would be maintained and moved to ensure survival. No management practices would be carried out along this pathway.

It is unlikely that the species will reproduce along the pathway. Both cuttings and whole plants could be moved along this pathway. As mentioned above, seeds are also traded via ecommerce.

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: No management practices would be carried out specifically on the species along this pathway. The species itself is the commodity.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: It is unlikely that the organism will enter the risk assessment area undetected via the pathway 'Ornamental purposes other than horticulture' as this is the deliberate movement of plant material into the risk assessment area.

See Qu. 1.6a for additional information.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: The species is traded and is available via the internet trade from both within and outside of the RA area. However, most stock would come from material already present within the RA area.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: When considering the pathways into the RA area, it is likely that *F. baldschuanica* can be introduced in the RA area with a medium confidence. All biogeographical regions would have similar likelihood scores based on the pathway described as the species would be imported for horticulture purposes. However, the species is probably not planted in areas unsuitable for species establishment characterised either by very low winter temperatures or very dry summer conditions.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Within the next 30/50 years, under the medium climate change prediction (RCP 4.5), when considering the pathways into the RA area, it is likely that *F. baldschuanica* will continue to be introduced in the RA area with a medium confidence as conditions will remain suitable for its planting. All biogeographical regions would have similar likelihood scores based on the pathway described as the species would be imported for horticulture purposes. Climate change is unlikely to change the current pathways but it may extend the areas where the species can be grown to the north and restrict the areas where the species may grow in the Mediterranean region.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[6] and the provided key to pathways[7].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name: (1) Horticulture (escape from confinement)

Qu. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	**medium**

Response: The pathway is the escape of the species from private gardens into the natural environment. Thus, this in its strictest definition would be an unintentional occurrence of the species in the environment outside of cultivation.

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment

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[6] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[7] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: There is no information on the volumes of movement along this entry pathway. The GB NNSS (2011) highlights the dispersal mechanisms of *F. baldschuanica* in Great Britain as: Most commonly, Russian-vine spreads vegetatively when thrown out from gardens. It readily survives uprooting and can spread through stem layering and root fragments. It also produces seed, though seedlings are relatively infrequent.

The species has a fast growth rate and can grow over the 'garden fence' over one or a few seasons. Espirito-Santo *et al* (2006) detail that its densely twining stems can grow up to 15 m long with a strong annual growth, making the stems long enough to potentially reach natural environments. The stems of *F. baldschuanica* can readily root in soil when fragmented from the main stem and produce new plants (pers. Obs. Tanner).

It should be noted that *F. baldschuanica* is often grown in urban settings and as the seed shows low viability, the species could only enter the natural environment by growing into it from a garden or fragments can be dumped or spread through management practices. In cities and other urban situations, it is assumed that most gardens are not directly bordering natural environments.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: It is considered as moderately likely with a medium uncertainty that *F. baldschuanica* will enter the environment undetected. Potentially, management methods of garden populations will fragment stems and lead to these fragments entering the natural environment.

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: If entering the natural environment from gardens, potentially through growth and /or management practices, this would be in the summer months which may be the months of the year most appropriate for establishment. Additionally, the autumn months with rainfall may promote the establishment of the species.

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: It should be noted that *F. baldschuanica* is often grown in urban settings and as the seed shows low viability, the species could only enter the natural environment by growing into it from a garden or fragments can be dumped or spread through management practices. Importantly, in cities and other urban situations, it is assumed that most gardens are not directly bordering natural environments.

The movement of transfer is likely to be limited (unless human mediated by the discarding of garden waste), but there remains a moderately likelihood of the species entering the natural environment from gardens. The low confidence reflects that organism transfer to natural environment is poorly documented and in a lot of situations, it is assumed the species will not reach the natural environment.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: It is moderately likely that the species can enter the environment within the risk assessment area based on the pathway Horticulture (escape from confinement).

Pathway name: (2) Landscape/flora/fauna "improvement" in the wild (escape from confinement)

Qu. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	**medium**

Response: The pathway is the escape of the species from amenity or landscaping plantings. For example in urban areas (buildings and fences) and areas of infrastructure such as roads and railways.

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: There is no information on the volumes of movement along this entry pathway.

See 2.3a for relevant information.

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: It is moderately with a medium uncertainty that *F. baldschuanica* will enter the environment undetected. Potentially, management methods of amenity or landscaping plantings will fragment stems and lead to these fragments entering the natural environment.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the

year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: If entering the natural environment from amenity or landscaping plantings, potentially through growth and /or management practices, this would be in the summer months which may be the months of the year most appropriate for establishment. Additionally, the autumn months with rainfall may promote the establishment of the species.

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: It should be noted that *F. baldschuanica* is often grown in urban settings and as the seed shows low viability, the species could only enter the natural environment by growing into it from a garden or fragments can be dumped or spread through management practices. Importantly, in cities and other urban situations, it is assumed that most amenity or landscaping plantings are not directly bordering natural environments.

The movement of transfer is likely to be limited (unless human mediated by the discarding of amenity or landscaping plantings waste), but there remains a moderately likelihood of the species entering the natural environment from gardens. The low confidence reflects that organism transfer to natural environment is poorly documented and in a lot of situations, it is assumed the species will not reach the natural environment.

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: It is moderately likely that the species can enter the environment within the risk assessment area based on the pathway ornamental other than horticulture pathway (escape from confinement).

Pathway name: (3) escape from confinement (to include dumping of plant waste from horticulture and ornamental purposes other than horticulture into the natural environment

Qu. 2.2c. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional	**CONFIDENCE**	**medium**

Response: The pathway is the release of the species from horticulture and ornamental purposes other than horticulture into the natural environment through dumping of plant material. Thus, this in its strictest definition would be an intentional entry of the species in the environment outside of planting. However; it could also be considered as unintentional if plant material is mixed with other waste or the owner expected it to compost away rather than establish.

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**Likely**	**CONFIDENCE**	**low**

Response: There is no information on the volumes of movement along this entry pathway.

The GB NNSS (2011) highlights that the species is thrown out from gardens. It readily survives uprooting and can spread through stem layering and root fragments. It also produces seed, though seedlings are relatively infrequent.

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**Likely**	**CONFIDENCE**	**medium**

Response: It is likely that *F. baldschuanica* will enter the environment undetected at the initial stage of release if plant material is mixed with plant material of other species.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: If dumping occurs in the spring or summer months, the species would be likely to establish. Dumping in the summer months is likely as this is when the growth of the species will be managed to prevent spread and the plant material could be discarded into the natural environment. Additionally, the autumn months with rainfall may promote the establishment of the species.

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: The dumping of the species is likely to be localized in the area where the plant is growing and plant material may be dumped directly in a habitat suitable for the species. As previously mentioned, the seed of *F. baldschuanica* have low levels of germination and thus. Seed is unlikely to be a contributing factor.

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: It is likely that the species can enter the environment within the risk assessment area based on the pathway **escape from confinement (**to include dumping of plant waste from horticulture and ornamental purposes other than horticulture into the natural environment.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response: It is moderately likely that the species can enter the environment within the risk assessment area based on the pathway horticulture and other ornamental purposes (escape from confinement).

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: When considering all pathways into the RA area, it is likely that *F. baldschuanica* can enter habitats within the RA area with a moderate confidence. Climate change may extend the areas where the species can be grown to the north and restrict the areas where the species may grow in the Mediterranean region. Both increased summer and winter temperatures would benefit the species. Increased precipitation and CO2 levels as a result of climate change may also favor the species. All biogeographical regions would have similar likelihood scores based on the pathways described.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: *Fallopia baldschuanica* has already established in the following countries: Belgium, Czech Republic, France, Great Britain, Ireland, Italy, Portugal, Romania, Slovakia, Spain. The species is established in the Atlantic, Continental and Mediterranean biogeographical regions and further establishment within the RA area is likely especially in areas where the species has been planted and these areas border natural environments.

There is no evidence available from scientific publications on the environmental requirements for *F. baldschuanica* and therefore information has been taken from the horticulture trade.

The RHS detail that *F. baldschuanica* can grow in full sun or partial shade. The species can grow in any poor to moderately fertile soil, which is moist but well drained.

The Missouri Botanical Garden (2019) detail *F. baldschuanica* can tolerate some drought.

EPPO (2012) detail that the species can tolerate temperatures down to -20°C, but is sensitive to long lasting periods of frost.

From observations in France, the species has been observed to persist for up to 10 years. G. Fried (personal observation, 2019) highlights: on a coastal dune near Sérignan-Plage, Hérault (FR), a stable population has persisted between 5-7 years. Again, in Hérault, in a riparian habitat, it has shown to climb on shrubs (Salix) and to be very persistent. In a 3rd site in the South of France, in wasteland where green garden waste was dumped, it has persisted for more than 10 years and forms a mat of more than 100m². The species is still present in all of these aforementioned sites in 2019.

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	**widespread**	**CONFIDENCE**	**high**

Response: *Fallopia baldschuanica* has been reported to grow in disturbed habitats, mainly as an escape from gardens in urban settings. Preston *et al*. (2002) details that the species rarely naturalizes away from urban areas. In Italy, the presence of naturalized populations is found mostly in disturbed areas, close to towns, along roads and road-verges, at the border of agricultural areas, in road slopes, and along railways. In the invaded sites it can reach high covering and climb on the surrounding vegetation. It is a rapidly growing creeper and has been described by Lousely and Kent (1981) as "rampant in hedgerows" in rural environments in the UK. In Belgium, the species has been recorded growing in sea dunes in Duinbergen (Knokke). NOBANIS also report the species growing in coastland habitats. In Austria, the plant has mainly been found on ruderal sites (building sites, along railway sites, roadsides) and in (semi-)natural habitats (wood edges, riverbank) (Essl and Stöhr 2006, Stöhr *et al*. 2012). In Spain, *F. baldschuanica* has been reported as colonising riparian sites (Sanz-Elorza *et al*. 2004).

All of the aforementioned habitats are widespread in the RA area.

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	**N/A**	**CONFIDENCE**	**high**

Response: *Fallopia baldschuanica* does not require another species for any stage of its lifecycle.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response: It is likely that *F. baldschuanica* will establish despite competition from existing species. However, a low confidence is given as it is not clear why the species is rarely observed in natural environments and if specific factors limit its development in these areas. *F. baldschuanica* can grow very quickly and smother other vegetation. The lack of natural enemies within the RA area provides the species with an advantage over native species.

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**low**

Response: *Fallopia baldschuanica* is native to Asia and thus co-evolved natural enemies would be present in this region and not within the risk assessment area. Those more generalist organisms naturally present in the risk assessment area, which might feed on the species, are unlikely to prevent the establishment of the species. However, it has been subject to infection by *Ustilago raciborskiana*, the agent of witches' broom in Italy (Verona and Bozzini, 1956) which can cause significant damage.

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	**moderately likely**	**CONFIDENCE**	**Medium**

Response: There are a number of management practices applied to *F. baldschuanica* within the risk assessment area. However, these management practices are mainly applied to established populations. Other areas, such as ruderal habitats and urban areas bordering natural environments may be overlooked and therefore provide habitats for establishment despite existing management practices.

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	**moderately likely**	**CONFIDENCE**	**Medium**

Response: The current urbanization trend occurring in Europe may favor the establishment of the species. Further development of urban gardens, developed in small spaces may favour the need for fast growing vine species which can be grown to increase privacy.

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	**moderately likely**	**CONFIDENCE**	**Medium**

Response: *Fallopia baldschuanica* is moderately likely to survive eradication campaigns. The species can be controlled with the manual removal of the stems though this can be difficult as the species can twist its vines around structures and other plants and thus care would be needed to remove all of the plant and all populations in the natural environment. Control may also be harder to achieve for this species as it has woody vines.

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**likely**	**CONFIDENCE**	**Low**

Response: Just one individual planted in an area where it could escape (grow into the natural environment) could be enough to form an established population. This is despite of the fact that seed viability within the RA area is reported to be limited.

The vigorous growth of the stems and the ability of the species to grow over structures and other plans can act to facilitate establishment of the species.

The wide tolerance of the species to sunlight/shade, soil requirements and moisture levels can act to facilitate the establishment of the species.

However, a low confidence is given as it is not clear why the species is rarely observed in natural environments and if specific factors limit its establishment in such areas.

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: There is no available information on the adaptability of *F. baldschuanica* apart from the information that it is tolerant to a wide range of abiotic conditions (see Qu. 3.9).

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	likely	CONFIDENCE	medium

Response: It is likely that *F. baldschuanica* can establish despite low genetic diversity in the founder population. The species can readily regrow from cuttings (or fragmentation) of the stems.

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	Likely	CONFIDENCE	medium

Response: The species is already established within the RA area. Further casual populations of *F. baldschuanica* are likely to occur within the RA area if the climatic and environmental conditions permit. *F. baldschuanica* is frequently planted in urban situations close to ruderal or semi-natural habitats and from these source populations, though fragmentation of the stems, plants can escape to form casual populations.

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	moderately likely	CONFIDENCE	low

Response: The species is already established within the RA area and the overall likelihood of establishment in the RA area is moderately likely. Although the species is planted throughout the RA area, the species is only established within the natural environment within a limited number of Member States. If the species is planted in areas with suitable climates, i.e. areas within the Atlantic, continental and Mediterranean biogeographical regions, further establishment may be seen.

The climatic conditions of the native range compared to that of the RA area are different. In China, and much of the native range, the Köppen Geiger climate classification type is Cfa and Cwa where only the former occurs in the RA area in Italy, Serbia Bulgaria and Romania.

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: Under a climate change scenario of (RCP 4.5, over the next 30/50 years), the Atlantic and Continental biogeographical regions are envisaged to remain suitable for the establishment of the species. Areas of the Mediterranean may become more limited for the establishment of the species due to increased temperature and more increased drought periods. With climate change, areas of the boreal, Black Sea and alpine biogeographical regions may become suitable for the establishment of the species. The Missouri Botanical Garden (2019) detail *F. baldschuanica* can tolerate some drought but it is not known to what levels of drought the species can withstand.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic “spread” via release or escape (“jump-dispersal”), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	**minor**	**CONFIDENCE**	**low**

Response: Spread of *F. baldschuanica* is mainly through physical extension of existing foliage from a single rootstock, growing up to 10m from that rootstock or from layering whereby connected plantlets allow further extension after contact with the soil. There are anecdotal reports on USA discussion fora of the species self-seeding readily, and being a “wildly rampant vine” (Dave’s Garden, 2009). A study on urban greening in China revealed that the plant can be easily propagated by seed, cuttings and layering, and thus is strongly recommended for urban greening (Guo et al, 2008) and they go on to state that success rate from seeds to seedlings is over 80%, following “proper propagation process”. This is in conflict with Tiebré *et al*. (2007) who reports low viability of seed collected in Belgium. As above, it is assumed that seed viability in the RA area is low.

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental

conditions in the Union.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Response: Once established in the environment it is relatively unlikely that intentional human assistance will be a prevalent means of spread, however inadvertent spread through soil movement and associated machinery is moderately likely. However, as there have been no scientific studies or case studies on the spread of the species by human assistance, a low rating for confidence has been given.

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name: UNAIDED (natural spread)

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	medium

Response: The spread of *F. balschuanica* via natural methods is the unintentional spread of the species within the RA area. There is little information on the natural spread of the species within the RA area and more globally, but the species has the potential to spread via seed and via the growth of the stems.

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**unlikely**	**CONFIDENCE**	**low**

Response: Natural spread is not regarded as a major spread mechanism for the species in the RA area. As previously mentioned, seed viability is considered low within the RA area and the species does not naturally produce stem fragments that can be spread within the environment. It is unlikely that birds or other small mammals will act to spread the species within the RA area.

There is no information on the rate of natural spread of the species in the RA area. It is highlighted through a number of gardening forums that the species can grow rapidly but this will only be from the parent plant.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: As previously highlighted, spread of *F. baldschuanica* is mainly through physical extension of existing foliage from a single rootstock, growing up to 10m from that rootstock or from layering whereby connected plantlets allow further extension after contact with the soil. Thus, even without viable seed in the population, there is the potential of new plants forming separate to the original root stock. However, when considering survival from cuttings, Greer (1976) notes that rooting was obtained in 3-5 weeks when cuttings, 0.5 cm and greater in diameter and including 2 nodes, were taken from dormant plants and inserted in a closed frame under high light intensity. During hardening-off and after potting up in individual containers, it was necessary to avoid low temperatures, reduced light intensity or excessive dryness, otherwise the cuttings became dormant. Thus, it would appear that cuttings are not very resilient, and this means of establishment may not be very effective.

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: In theory, the species should be relatively straight forward to manage but stem fragments that have rooted within soil may potentially be missed during management practices. These stem pieces could remain in the environment and generate into new plants.

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**unlikely**	**CONFIDENCE**	**medium**

Response: Given that most spread via natural means would be due to the growth of the stem from the parent plant and the potential rooting of stem fragments, it is unlikely that spread within the RA area would go undetected.

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**likely**	**CONFIDENCE**	**moderate**

Response: *F. baldschuanica* is mainly planted in urban habitats and escapes from these areas where it can transfer to suitable habitats. Examples of this include the presence of the species in hedgerows in rural environments in the UK (Lousely and Kent, 1981) and sea dunes in Belgium and riparian sites in Spain (Sanz-Elorza *et al*. 2004).

As planting of the species either as horticulture planting or other ornamental uses often occurs close to suitable natural habitats, it is likely that the species can transfer to a suitable habitat with a moderate uncertainty.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**very slowly**	**CONFIDENCE**	**low**

Response: The overall potential rate of spread via this pathway is estimated as very slowly with a low confidence. A low confidence has been given as it is difficult to quantify natural spread as dynamic distribution maps often integrate both natural spread and human-assisted spread.

Pathway name: Transport – contaminant (transport of habitat material (soil, vegetation)

Qu. 4.3b. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: There is unlikely to be any intentional movement of this vegetation through the movement of habitat material (soil and vegetation). The spread pathway considered in this section is the unintentional movement of seed and plant fragments through the transport of habitat material (soil and vegetation).

Qu. 4.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: It would only take a very low number of individuals (stems) to establish a new population given the ability of the plant to establish in relatively harsh conditions. However, these stems would need to root and form a viable plant within the substrate.

Qu. 4.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: The pathway is likely to involve relatively short periods of movement and therefore there would be little chance to reproduce. However, if the plant material is rootstock or stem material it is likely that these potentially reproductive plant parts would remain viable during transport

Qu. 4.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: There are generally no management practices applied on this pathway and there would not be any specific inspection practices for plant parts of *F. baldschuanica.*

Qu. 4.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: The plant is quite obvious once it grows so it is likely to be detected. However, during the initial phase, if viable stem fragments are spread there is the potential that these plant parts will not be detected until the plant forms an established population.

Qu. 4.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Soil is what the plant needs to survive along with water and other common abiotic inputs and the movement of soil, either in bulk or other habitat material, is the subject of this pathway.

Qu. 4.9b. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**very slowly**	**CONFIDENCE**	**low**

Response: Only a few plants are established in the wild in Europe and it would need movement of soil from these sites to facilitate spread which would be limited

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	**with some difficulty**	**CONFIDENCE**	**low**

Response: There is no direct assessment available for this but buried material is hard to detect in soil so preventing its unintentional spread and containing it would be hard of the movement of soil continues.

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	**slowly**	**CONFIDENCE**	**low**

Response: the plant is not currently widespread in the natural environment and often it exists as single plants or limited populations in number rather than scale, so the rate of spread has been slow and is unlikely to be anything but slow in the future.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	**slowly**	**CONFIDENCE**	**medium**

Response: *F. baldschuanica* is tolerant of many varied conditions and is not seriously limited by climate in the RA area. Climatic changes will allow it to establish further north than present but its inherent rate of spread should remain low.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comment: There are no specific studies in the non-native range outside the RA area that specifically assess impacts on biodiversity. *F. baldschuanica* can climb very rapidly, growing over shrubs and trees and this smothering behavior can act to rapidly out-compete plant species for light and space.

Within its non-native range, *F. baldschuanica* is presumed to be host very few phytophagous natural enemies so the local impact on biodiversity could be high as the species remains unregulated by such natural enemies compared to native plant species. However, this is likely to be limited spatially as it can only grow up to 10m away from its rootstock.

If an invasive population was to appear in a very sensitive habitat it could lead to local suppression or extinction of native plants and/or their associated specialist natural enemies however, it is likely that such an invasion would be detected as part of the sensitive habitat monitoring programme before such impacts could be felt.

It should be highlighted that *F. baldschuanica* is rarely found in areas of conservation value, mainly the species is found in ruderal urban habitats. Therefore, the impact on biodiversity is likely to be minor with a low confidence.

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comment: There is limited information on the impact of the species, where most are observations on the mat forming and smothering behavior of the species where it escapes from planted situations. There are no known scientific studies that have evaluated the impact of the species on biodiversity and thus the 'known impact' can only be assessed as moderate.

Brunel et al (2010) suggest that Russian vine has a high impact on the environment but with high uncertainty. This reflects perceptions in the field from observations on invasions but without the scientific rigour or published papers to give any significant level of confidence.

Brunel *et al.* (2010) citing Sanz Elorza *et al.* (2004) states "this vine grows over shrubs and trees, and along riparian forests [in Spain].

From observations in France, the species has been observed to persist for up to 10 years. G. Fried (personal observation, 2019) highlights thaton a coastal dune near Sérignan-Plage, Hérault (FR), a stable population has persisted between 5-7 years. Again, in Hérault, in a riparian habitat, it has shown to climb on shrubs (Salix) and to be very persistent.

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comment: In the future e.g. 2070, the number and scale of invasive populations may increase due to increased planting resulting from more urbanization and increased residence time which may enhance establishment capacity. However, this is only expected to raise from minor to moderate. This could potentially shift the biodiversity impact to a higher level but again without the peer-reviewed literature to support this expectation, confidence is low.

Qu. 5.4. How important is decline in conservation value with regard to European and national

nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comment: Though *F. baldschuanica* is able to colonise a wide array of habitats some with conservation potential. For examples, it is a rapidly growing creeper and has been described growing in hedgerows in rural environments in the UK Lousely and Kent (1981), sea dunes in Duinbergen (Knokke) Belgium. NOBANIS (2019) also report the species growing in coastland habitats. In Spain, *F. baldschuanica* has been reported as colonising riparian sites (Sanz-Elorza *et al*. 2004). In these areas, as with the whole of the EU, there are no records of it having a negative impact on the conservation value of the habitat. Thus, the importance of a decline in conservation value of habitats within the RA area is minor.

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comment: In the future, the number and scale of invasions by *F. baldschuanica* is likely to increase but it is likely that invasions in sensitive habitats will be detected and eradicated before it can have a significant detrimental impact. The species does not have underground rhizomes that will facilitate spread and thus eradication would be easier than that of *F. japonica*, for example.

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comment: *F. baldschuanica* has been promoted by the horticultural trade as an excellent screening plant and as such it has been widely planted in gardens. Once established, its rampant growth can get out of control, causing disputes between neighbours. It can also restrict visibility for road users (GB NNS datasheet http://www.nonnativespecies.org/factsheet/downloadFactsheet.cfm?speciesId=1493). Therefore, it could be considered to have some cultural impact.

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	**minimal**	**CONFIDENCE**	**low**

Comment: There are no data on the impact of *F. baldschuanica* on ecosystem services within the RA area. The authors suggest that the impacts are likely to be negligible.

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	**minimal**	**CONFIDENCE**	**low**

Comment: There are no data on impacts of the species but the impacts are likely to be minor. The species may be used more in the future in urban situations, but it is unlikely this will cause greater impacts.

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comment: It is evident from online discussion in the USA that some homeowners spend a lot of time trying to manage and/or eradicate *F. baldschuanica* infestations but there are no data on actual damage costs. *F. baldschuanica* can damage power and telephone lines, trees, shrubs and boundary structures due to its sheer biomass (GB NNSS, 2011) and the smothering habit of the plant. Potentially, these types of damage could be significant. Nonetheless, it is likely that the global cost of Russian Vine is moderate.

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comments: There are no specific data on economic impacts in the RA area but it is likely that damage costs from impacts like broken fences and damaged power lines across Europe are more than 10,000 euros so the impact is likely to be minor. A low confidence is given due to the lack of information on this subject.

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of

management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comments: It is likely that costs will increase, potentially due to the increase in use of the species in horticulture. However, the increase is not likely to be significant and not enough to become moderate

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comments: No information has been found on the issue but it is likely that the costs associated with management by land owners in the RA area is not insignificant and could be estimated as minor.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comments: No information has been found on the issue but it is likely that the costs associated with future management by land owners in the RA area is not insignificant but may not increase beyond the category of minor.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of

people, property or infrastructure;

- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**minimal**	**CONFIDENCE**	**low**

Comments: There are no direct or indirect human health issues recorded for this species apart from the potential for road accidents due to it restricting visibility for road users. However there may be some social impacts since, once established, its rampant growth can get out of control, causing disputes between neighbours. (GB NNS datasheet http://www.nonnativespecies.org/factsheet/downloadFactsheet.cfm?speciesId=1493)

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	**minimal**	**CONFIDENCE**	**low**

Comments: Though the likelihood of social and human health impacts will increase it is not anticipated that it will become a significantly more serious issue.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	**minimal**	**CONFIDENCE**	**medium**

Comments: There are no records of any of these impacts

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	**minimal**	**CONFIDENCE**	**medium**

Comments: There are no records of any of these impacts

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comments: There are relatively few natural enemies recorded in the invasive range apart from *Ustilago raciborskiana*, the agent of witches' broom in Italy (Verona and Bozzini, 1956) which can cause significant if localized damage.

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comment: Impacts are mainly restricted to the household border situation where damage, control and social costs are likely to be occasional and minimal. Impacts are likely to decrease with increasing distance from conurbations and these impacts will be local to the few established plants.

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comment: The overall impact in RA area in foreseeable climate change conditions is unlikely to change from that of the current climatic conditions. New (northern) areas of the RA area may be suitable for the establishment of the species but it is likely that the impact will remain minimal with a low confidence to reflect the uncertainty of both climate change prediction and the lack of scientific information on impacts. Areas in the Mediterranean may become less suitable for the establishment of the species in the natural environment and thus less impact may be seen in these areas.

RISK SUMMARIES

	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction*	**likely**	**medium**	When considering all pathways into the RA area, it is likely that *F. baldschuanica* can enter the region with a medium confidence. The species can enter via the horticultural trade pathway but much of the stock may come from within the RA area rather than from outside.
Summarise Entry*	**likely**	**medium**	When considering all pathways for entry into the RA area, it is likely that *F. baldschuanica* can enter the natural environment, with a medium confidence. Most populations are grown within urban habitats and the species is not commonly found within the natural environment.
Summarise Establishment*	**moderately likely**	**low**	Seed viability is considered as low within the RA area and establishment by stem fragments can occur but is restricted to suitable habitats close to the parent plant.
Summarise Spread*	**slowly**	**low**	Once established spread by natural means is likely to be slow as seeds are not a significant means of spread in the RA area. Human agency may assist spread but not as much as for establishment.
Summarise Impact*	**minor**	**low**	Impacts are mainly restricted to the household border situation where damage, control and social costs are likely to be occasional and minor. Impacts are likely to decrease with increasing distance from conurbations. There is no published information on the impact of the species in natural habitats within the RA area.
Conclusion of the risk assessment (overall risk)	**low**	**low**	Impacts on biodiversity and ecosystem services are likely to be low within the RA area. The species is grown within urban settlements and it rarely forms dense established populations within areas of high conservation potential.

*in current climate conditions and in foreseeable future climate condition

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Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	YES		YES	YES	
Belgium	YES	YES	YES	YES	
Bulgaria	YES	YES	YES	YES	
Croatia	YES	YES	YES	YES	
Cyprus			YES	YES	
Czech Republic	YES	YES	YES	YES	YES
Denmark	YES	YES	YES	YES	
Estonia			YES	YES	
Finland			YES	YES	
France	YES	YES	YES	YES	
Germany	YES	YES	YES	YES	
Greece	YES		YES	YES	
Hungary	YES		YES	YES	
Ireland	YES	YES	YES	YES	YES
Italy	YES	YES	YES	YES	YES
Latvia			YES	YES	
Lithuania			YES	YES	
Luxembourg			YES	YES	
Malta			YES	YES	
Netherlands	YES		YES	YES	
Poland	YES	YES	YES	YES	YES
Portugal			YES	YES	
Romania	YES	YES	YES	YES	
Slovakia			YES	YES	
Slovenia	YES	YES	YES	YES	YES
Spain	YES	YES	YES	YES	YES
Sweden			YES	YES	
United Kingdom	YES	YES	YES	YES	YES

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	YES		YES	YES	
Atlantic	YES	YES	YES	YES	YES
Black Sea			YES	YES	
Boreal			YES	YES	
Continental	YES	YES	YES	YES	YES
Mediterranean	YES	YES	YES	YES	YES
Pannonian			YES	YES	
Steppic			YES	YES	

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[8]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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[8] Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al*. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy

			Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[9]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires*

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[9] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			etc.
		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences

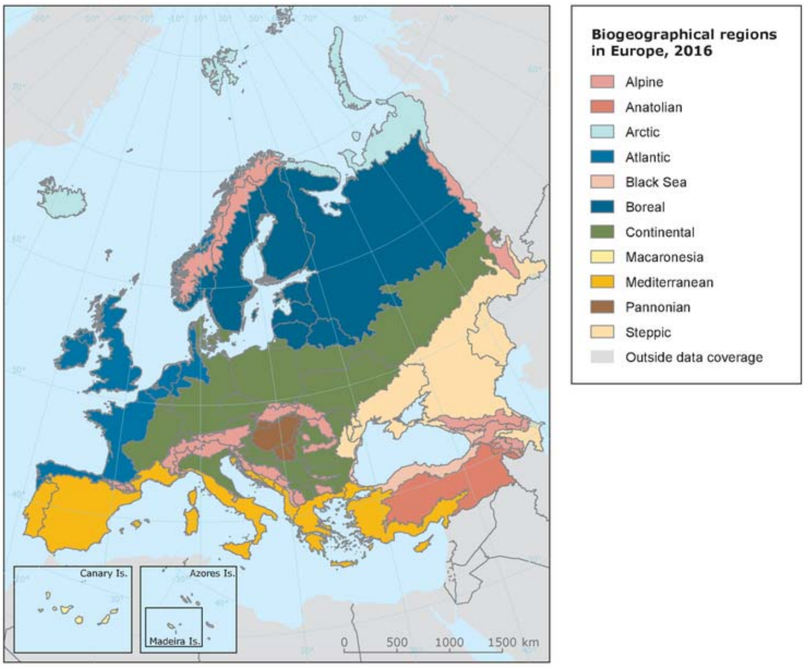
			Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

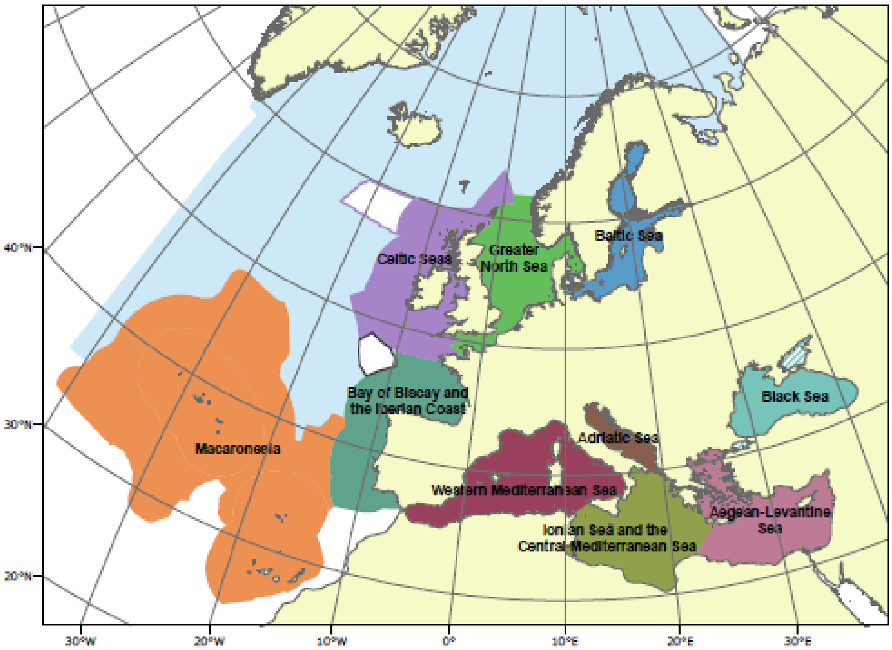
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

ANNEX VII Projection of climatic suitability for *Fallopia baldschuanica* establishment

Björn Beckmann, Rob Tanner, Richard Shaw, Beth Purse and Dan Chapman

30 October 2019

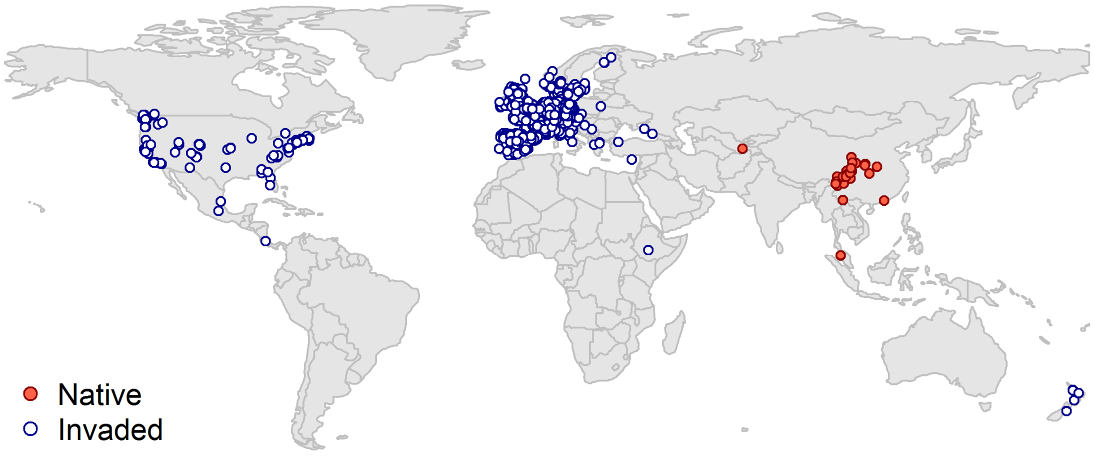
Aim

To project the suitability for potential establishment of *Fallopia baldschuanica* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (4074 records), iNaturalist (103 records), the Biodiversity Information Serving Our Nation database (BISON) (101 records), the Integrated Digitized Biocollections (iDigBio) (53 records), the Berkeley Ecoinformatics Engine database (9 records), and a small number of additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils, captive records) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 1175 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Tracheophyta records held by GBIF was also compiled on the same grid (Figure 1b).

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)

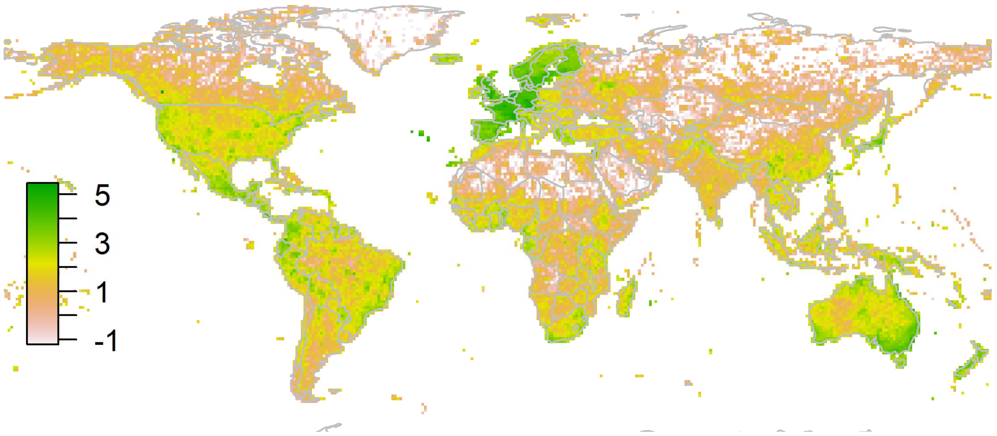


Figure 1. (a) Occurrence records obtained for *Fallopia baldschuanica* and used in the modelling, showing native and invaded distributions. (b) The recording density of Tracheophyta on GBIF, which was used as a proxy for recording effort.

Climate data were selected from the ‘Bioclim’ variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Fallopia baldschuanica*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Climatic moisture index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed. For its calculation, monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar radiation using the simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. There represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.

Species distribution model

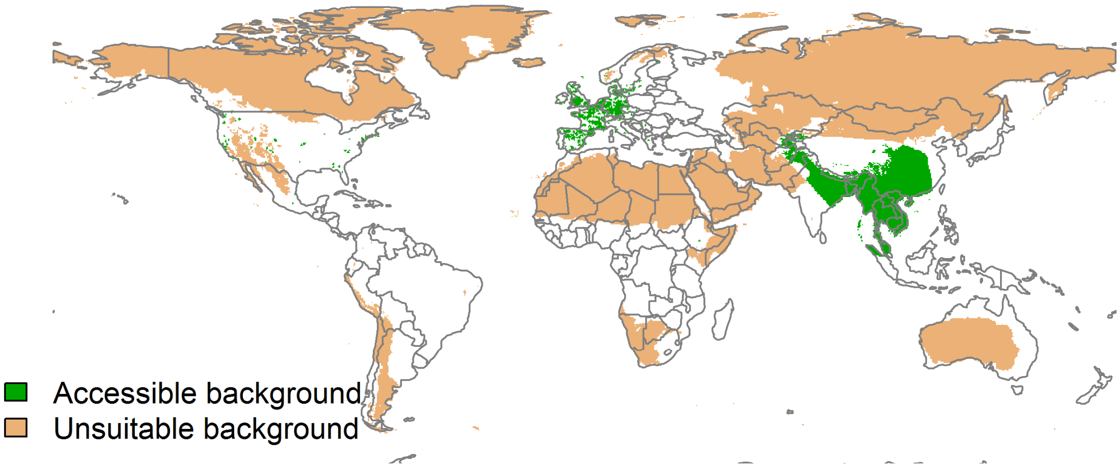
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1 (Thuiller et al., 2019, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Fallopia baldschuanica* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Fallopia baldschuanica* at the spatial scale of the model:
  - Minimum temperature of the coldest month (Bio6) < -19
  - Mean temperature of the warmest quarter (Bio10) < 8.5
  - Climatic moisture index (CMI) < log1p(0.25)

Altogether, only 0.3% of occurrence grid cells were located in the unsuitable background region.

Within the background region, 10 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Fallopia baldschuanica*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2’s default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen’s Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa’s dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the ‘minROCdist’ method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Fallopia baldschuanica* was most strongly determined by Minimum temperature of the coldest month (Bio6), accounting for 40.3% of variation explained, followed by Human influence index (HII) (32.3%), Climatic moisture index (CMI) (17.7%) and Mean temperature of the warmest quarter (Bio10) (9.7%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

					variable importance (%)			
Algorithm	AUC	Kappa	TSS	Used in the ensemble	Minimum temperature of the coldest month (Bio6)	Human influence index (HII)	Climatic moisture index (CMI)	Mean temperature of the warmest quarter (Bio10)
GLM	0.908	0.578	0.802	yes	37	42	9	12
GAM	0.907	0.576	0.805	yes	36	42	13	9
ANN	0.923	0.585	0.809	yes	45	29	12	15
GBM	0.917	0.580	0.807	yes	35	41	20	4
MARS	0.916	0.580	0.807	yes	52	17	29	2
RF	0.871	0.572	0.804	no	42	21	27	10
Maxent	0.916	0.583	0.809	yes	36	23	24	17
Ensemble	**0.916**	**0.583**	**0.811**		**40**	**32**	**18**	**10**

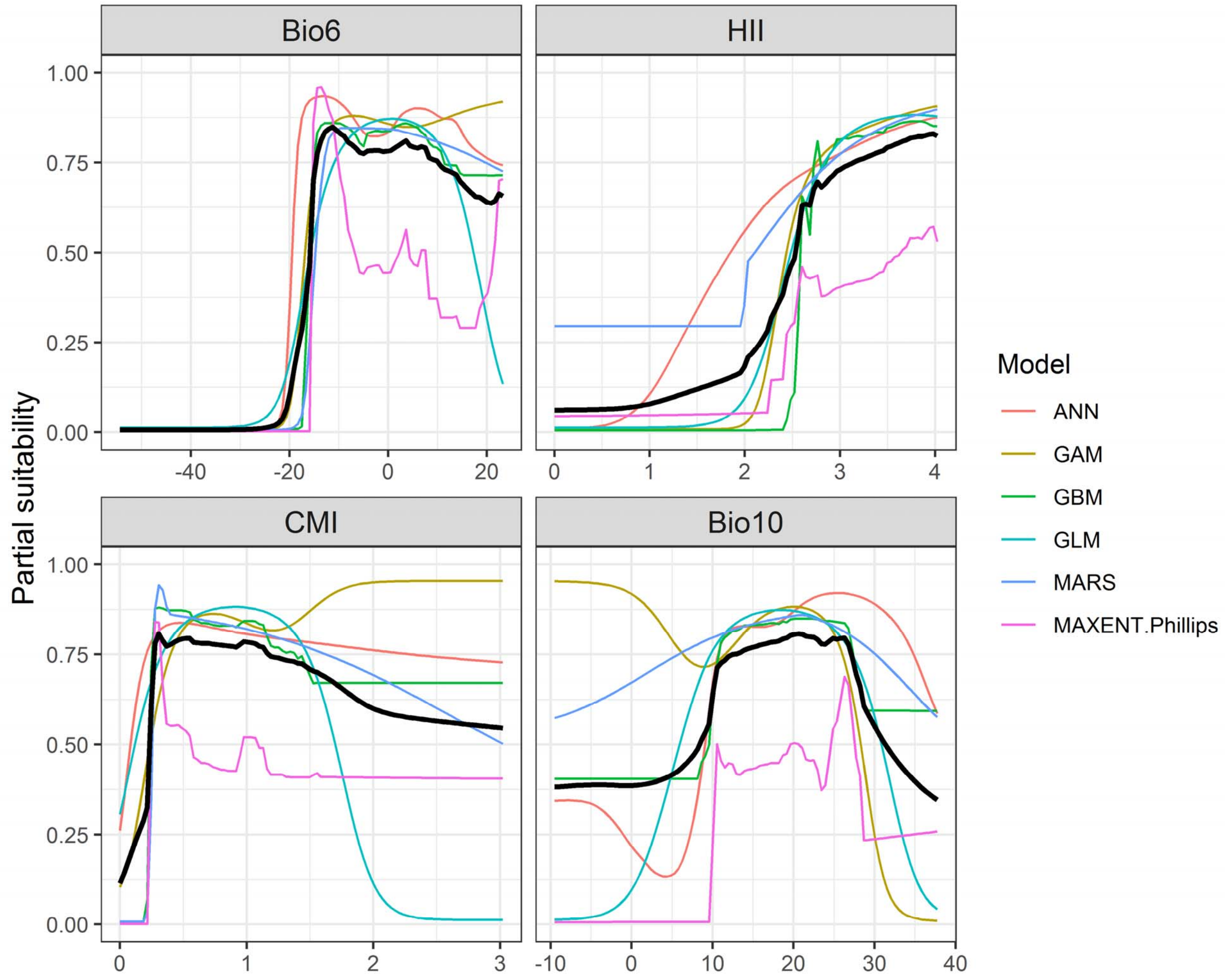
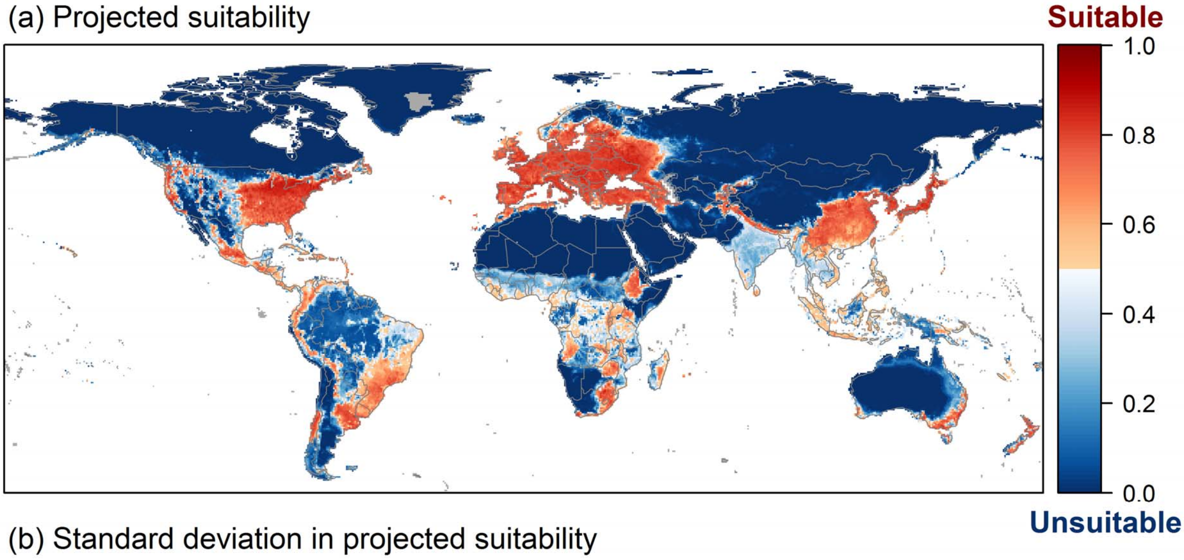


Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

(a) Projected suitability



(b) Standard deviation in projected suitability

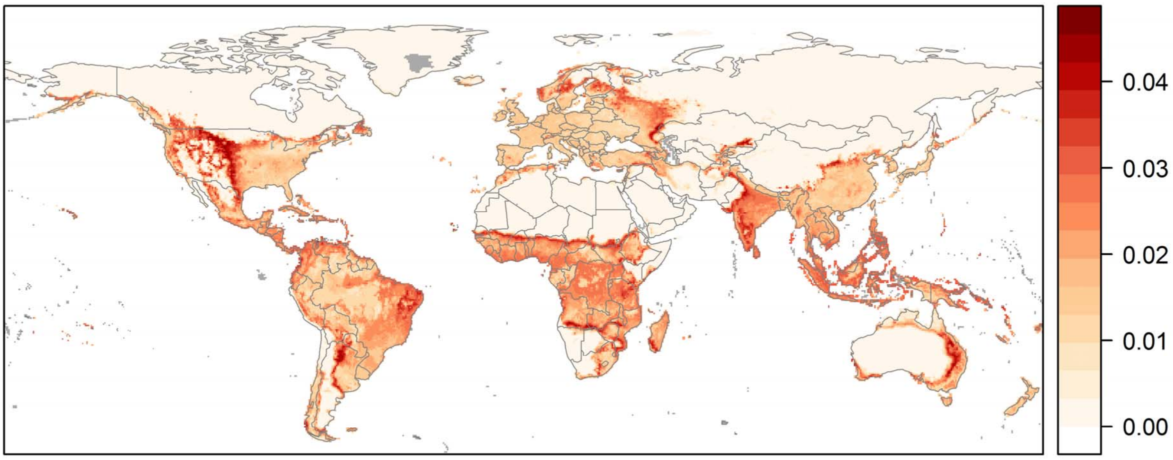
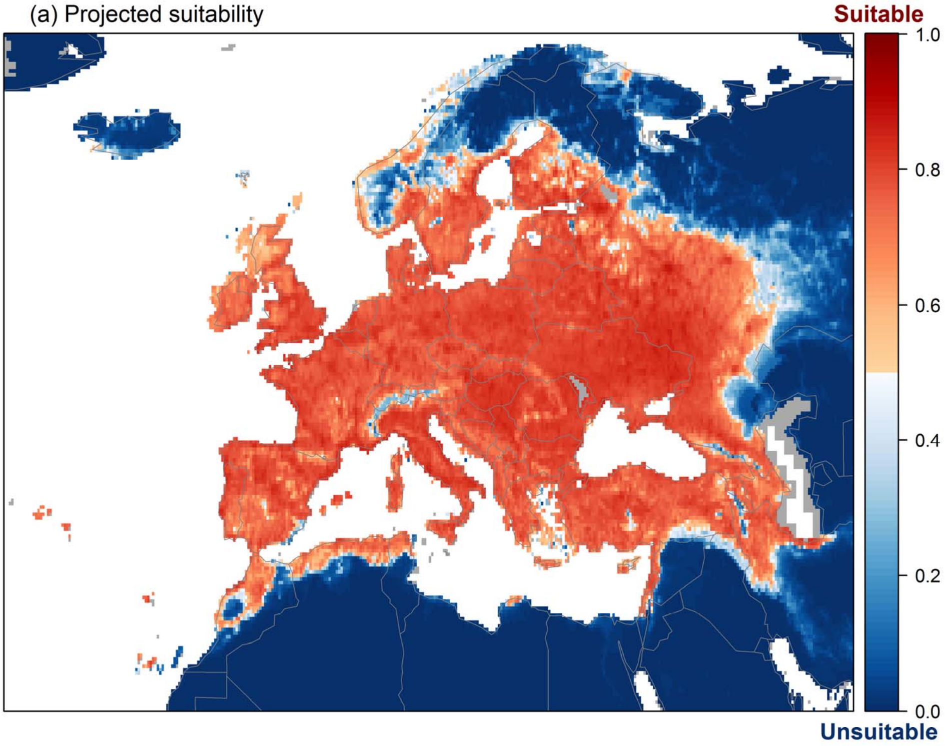


Figure 4. (a) Projected global suitability for *Fallopia baldschuanica* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.5 may be suitable for the species. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

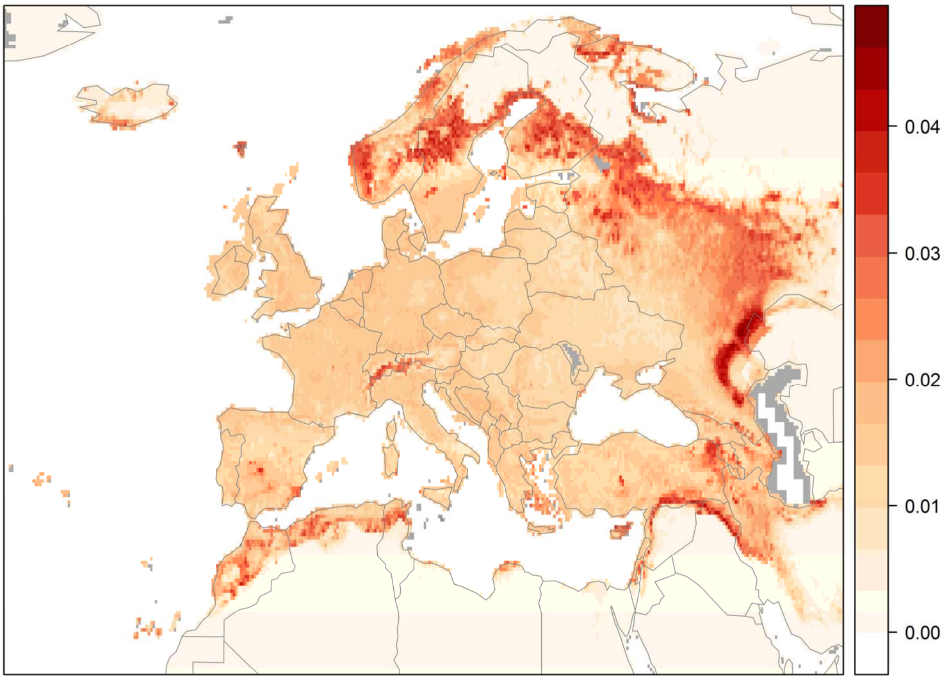


Figure 5. (a) Projected current suitability for *Fallopia baldschuanica* establishment in Europe and the Mediterranean region. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

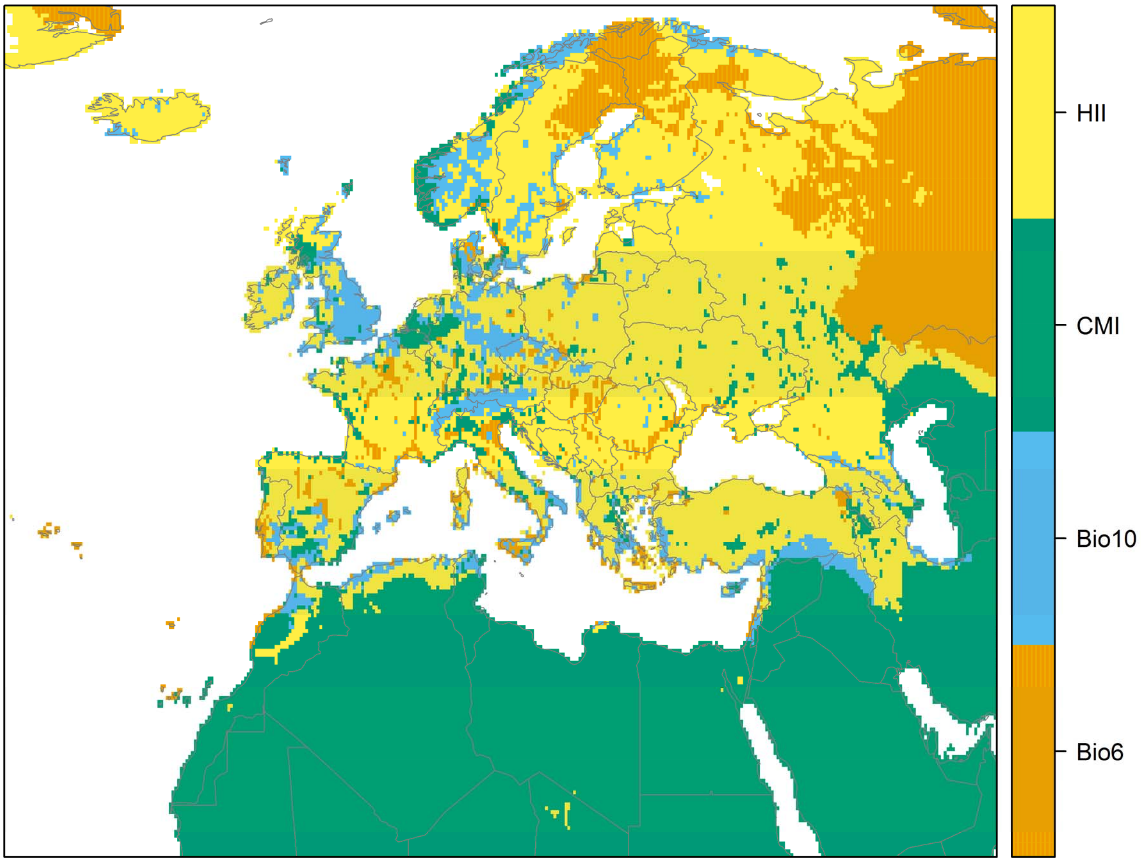
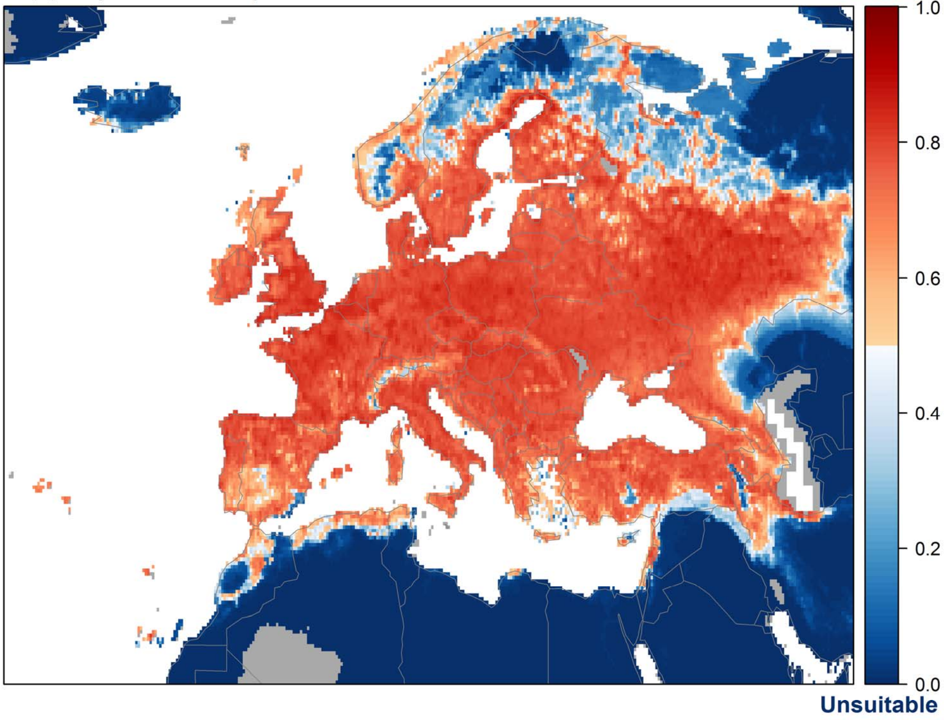


Figure 6. The most strongly limiting factors for *Fallopia baldschuanica* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.


(a) Projected suitability

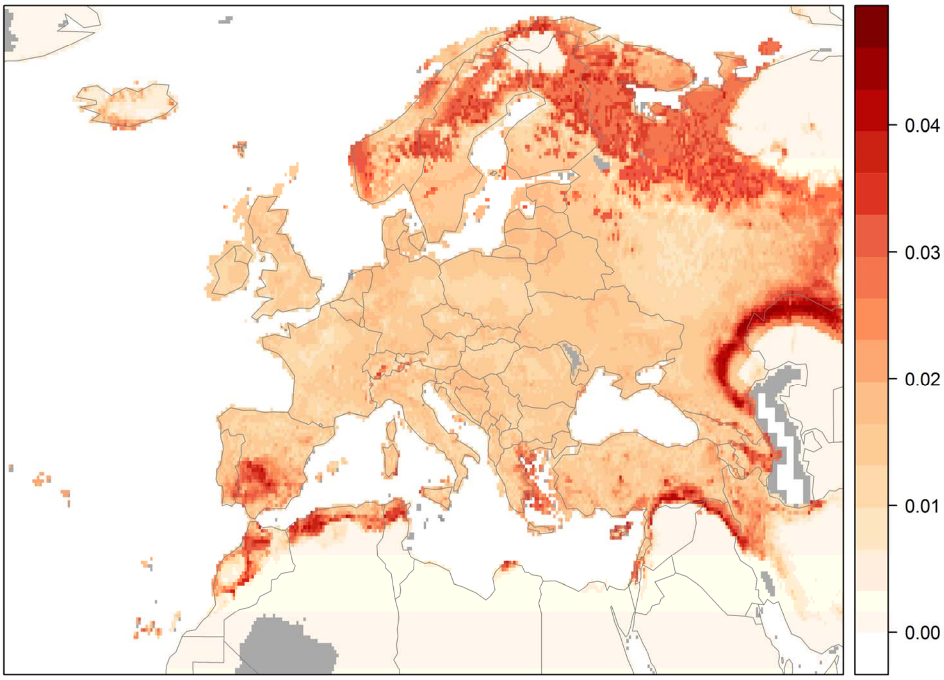
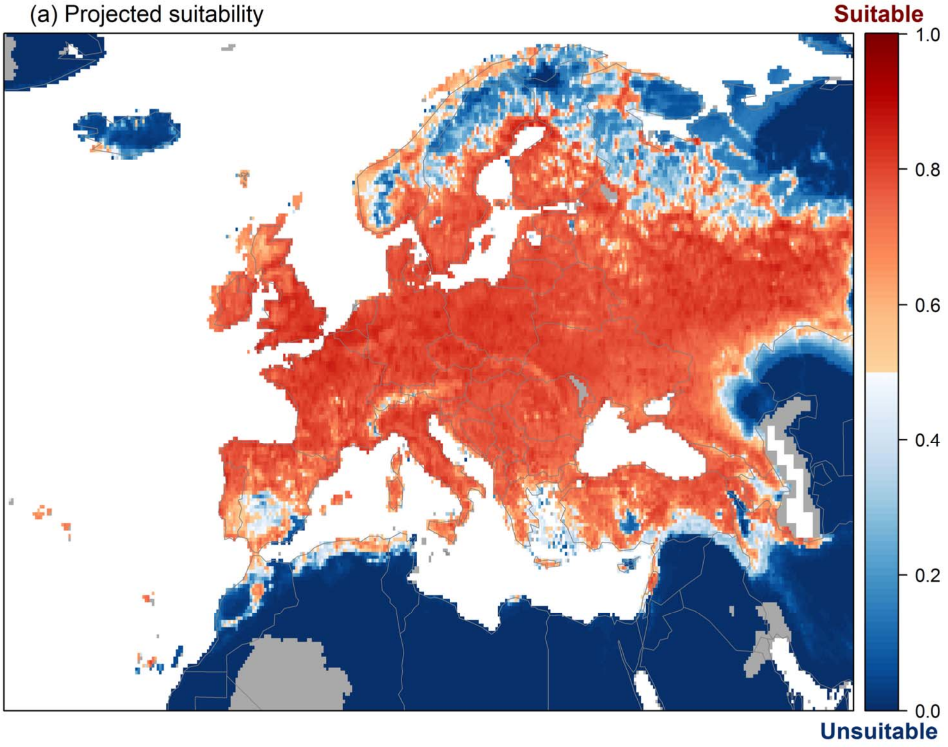

(b) Standard deviation in projected suitability

Figure 7. (a) Projected suitability for *Fallopia baldschuanica* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.


(a) Projected suitability

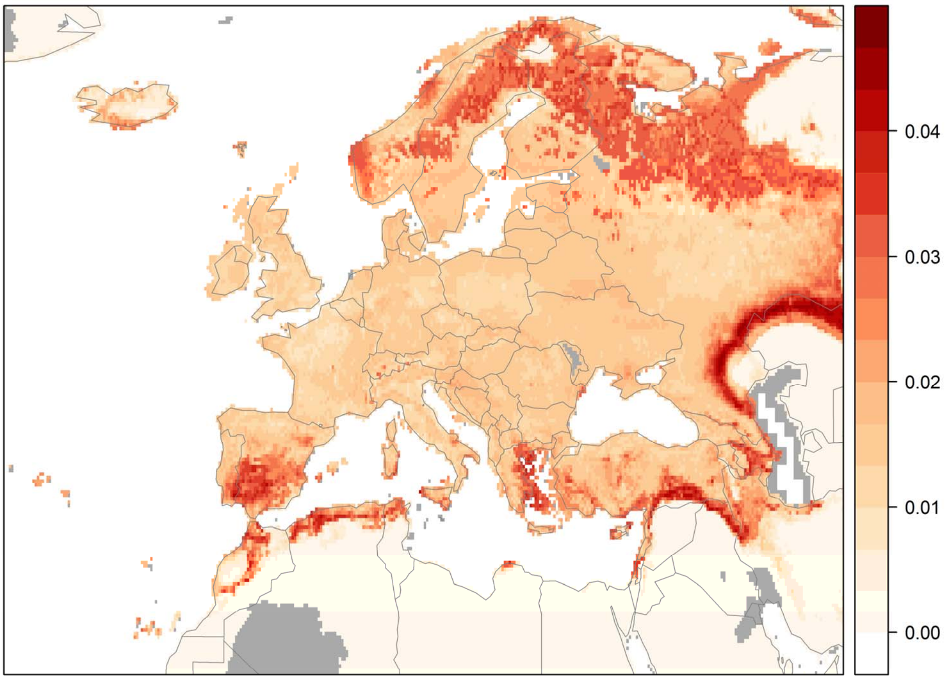

(b) Standard deviation in projected suitability

Figure 8. (a) Projected suitability for *Fallopia baldschuanica* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the

projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

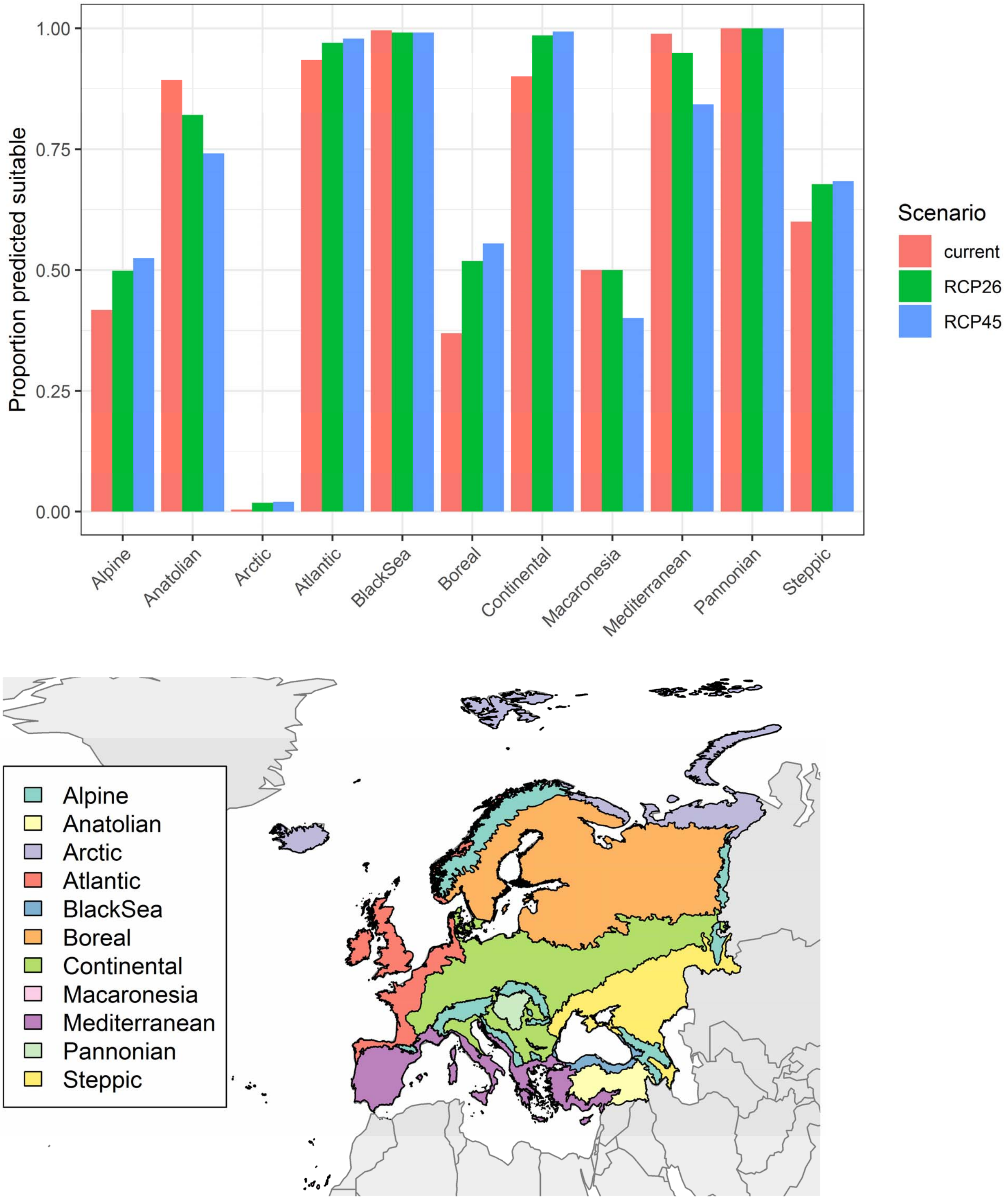


Figure 9. Variation in projected suitability for *Fallopia baldschuanica* establishment among Biogeographical regions of Europe (Bundesamt für Naturschutz (BfN), 2003). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected

climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

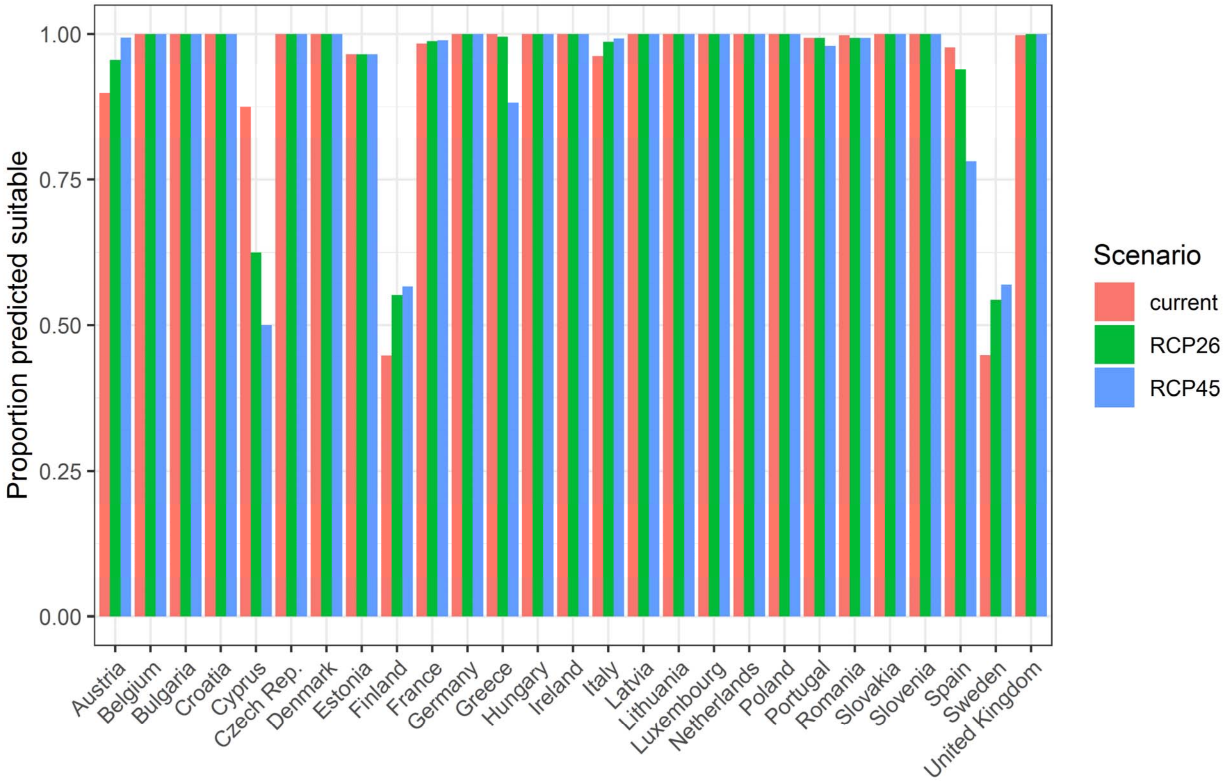


Figure 10. Variation in projected suitability for *Fallopia baldschuanica* establishment among European Union countries. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample was weighted by the density of Tracheophyta records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Fallopia baldschuanica*** **(Regel) Holub**
Species (common name)	**Russian vine**
Author(s)	**Rob Tanner**
Date Completed	**5.07.2019**
Reviewer	**Pete Robinson**

Summary [1]

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Fallopia baldschuanica **is already established within the risk assessment area and although the pathway of horticulture (escape from confinement) is detailed in the RA, the overall likelihood of the species entering via these pathways is moderate. Thus, to mitigate the impact of this species within the RA area, measures should focus on early detection and eradication of the species where it occurs.**

Although there are no specific studies on the management of ***F. baldschuanica*** **in the EU, it can be considered that management practices should follow that of other perennial vine species.** ***F. baldschuanica*** **can be managed with traditional methods including the use of physical and mechanical methods, and the utilisation of chemical control methods. However, the latter should be used with caution as** ***F. baldschuanica*** **often grows over other vegetation and chemical application is often non-selective. Physical and mechanical methods can include the utilization of various machinery and tools (strimmers, shears etc) and the physical pulling of rooted stems.**

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)**[3]	**Level of confidence**[4]
Methods to achieve prevention [5]	One potential pathway for entry or spread of *Fallopia baldschuanica* into the EU, or between Member States, is through the purchase of material through the horticultural trade. A ban from sale would help to regulate this pathway for the species. The species is also traded between Member States via internet suppliers.	*Fallopia baldschuanica* is traded within the RA area and grown as a garden ornamental. The species could be regarded as being a moderately popular species in trade and therefore a ban on the sale of the species would be a reasonably cost-effective measure at preventing the movement, both from outside and within the RA area. A ban from sale requires resources including financial resources, staff time and the development of communication material from a number of sectors, including governmental, regulators, horticulture and horticultural suppliers, the general public, and environmental NGOs. Communication material detailing the negative impacts of the species would be essential to educate the public and support a ban on sale. Public awareness campaigns may highlight the risk of the species and prevent further spread of the species from existing populations. Public awareness campaigns should highlight that fragments of the species should not be dumped in waste piles or natural areas (Kings County, 2019). Where possible campaigns should highlight alternative species that could be used. It is estimated that the cost for an awareness raising campaign could be up to EUR 10,000 per year (which would include the cost to produce and disseminate information material along with associated staff costs) for each Member State.	Moderate confidence in the assessment
	The transport of stem material as a contaminant of habitat material (soil and vegetation) may facilitate its spread within the EU. Phytosanitary inspections along with associated phytosanitary measures can act to prevent the	Phytosanitary inspections can be implemented on commodities coming into the EU from outside but the risk of *F. baldschuanica* entering as a contaminant is moderate as the seed is often not viable and stem material would be visible. The author could not find any examples where stem material has been intercepted as a containment.	Moderate confidence in the assessment

	entry of the species into specific countries/regions. To prevent the movement of contaminated material between EU Member States, management plans for habitat material, identification guides, factsheets, Codes of conduct should be referred too/developed. Preventing the spread of *F. baldschuanica* should also be regarded as a priority to limit further invasive populations. Measures to achieve this are listed in the eradication and management sections. Preventing the establishment of *F. baldschuanica* should be the priority as eradication can be difficult and complicated with limited options available.	It is however, very difficult to implement phytosanitary measures within the EU due to freedom of movement of commodities between countries. Therefore, this limits the effectiveness of inspections regarding the spread of the species within the EU. If measures are not implemented by all countries, they will not be effective since the species could spread from one country to another. National measures should be combined with international measures, and international coordination of management of the species between countries is recommended.	
Methods to achieve eradication [6] There are a number of methods that can potentially achieve eradication of discrete populations. However, if the population occurs along riverbanks or over a large area eradication attempts may be limited or will require investment over a number of years. It is also	**Manual control using mechanical or manual removal**	Mechanical and manual control can take the form of cutting using basic hand-held non-motorised utensils or motorised machinery such as strimmers sheers or other cutting devices. All plant material should be removed and safely disposed of following cutting. However, just cutting the vines will not control the plant as it can regrow after pruning. Instead, the main roots that connect to the soil should be severed. Physical removal is difficult as the plant can twist around plants and structures (Kings County, 2019). Handpulling of the stems is not recommended to achieve eradication of the species. Hand pulling can be used to remove the aboveground material. If the substrate is compact, handpulling should take place during time of rain	Moderate confidence in the assessment

important to note that any eradication method may need to be used in combination, for example removal of the above-ground foliage will not achieve eradiation alone and will need to be used in combination with cutting of the stems that connect to the soil substrate. Eradication methods can be applied on a local scale. Methods would depend on the habitat where the species is invasive and the extent of the infestation.		to facilitate the removal of the root system. Hand pulling may only be effective on small plants as once plants have established, they can develop an extensive root system. Such measures may be effective at removing the above ground biomass of the species, but would be relatively ineffective unless the stems that connect to the soil are cut. EPPO (2012) highlight that the only management method which has shown some effectiveness is the manual removal of plants though this can only be effective if subterranean organs are removed. In urban and ruderal habitats, management of the species using this method may be more effective than natural habitats where the species has twisted around natural vegetation. Costs will vary depending on the area infested and the type of habitat. If plant material is on a discrete piece of land, control costs could be as little as 100- 500 Euros. However, where the species invades a site with multiple landownership and/or if the species is growing over native vegetation, costs may be increased.	
	Chemical application (herbicides)	There is mixed information on the chemical control of the species. A systemic herbicide could be applied via a paint brush on the leaves, for example glyphosate. It is not recommended to spray herbicide on the foliage in urban areas where the species is growing and attached to other plant species as herbicides are non-selective.	Moderate confidence in the assessment
	Removal of root material	EPPO (2012) highlights that effective control should include the removal of subterranean organs. However, the roots are not like the rhizomes of Fallopia japonica and thus are not considered to facilitate the spread to a high extent. This may be practical difficult especially where the species has spread into adjoining land. Depending on the size of the infestation and the complexity of excavating the root stock, costs may be as little as 100- 500 Euros.	Moderate confidence in the assessment

Methods to achieve management [7]		See methods 1-3 in 'methods to achieve eradication' which can all be used to achieve management.	
Management methods can be applied on a local scale. Methods would depend on the habitat where the species is invasive and the extent of the infestation.	**Manual control**	Manual control can be used to control the spread of the species simply by pruning the growing stems of the plant. Additionally, cutting the tap root of the species and removing the roots can act to more permanently control the population.	Moderate confidence in the assessment
	Chemical control	Chemical control is probably not a cost-effective method for this species but it could be useful when controlling small populations of the species in discrete urban situations. Repeated and targeted applications may be needed to maintain suppression of the population.	Moderate confidence in the assessment
	Excavation of the roots	Excavation of roots from the soil, and contaminated soil may be cost effective over a long period of time but the initial outlay of costs can be high and include costs of heavy machinery and costs of disposal of contaminated soil	Moderate confidence in the assessment
	Biological control	At present, biological control against *F. baldschuanica* has not been considered as the extent of the problem the species causes is unlikely to warrant the investment. The cost-effectiveness of instigating and delivering a classical biological control programme against this species would initially be low as considerable costs would be needed to fund the control programme. A classical biological control programme can cost in the region of 600,000 EUR.	High confidence in the assessment

References

EPPO (2012) Mini data sheet on *Fallopia baldschuanica*. https://gd.eppo.int/taxon/BIKBA/documents

Kings County (2019) https://www.kingcounty.gov/services/environment/animals-and-plants/noxious-weeds/weed-identification/silver-lace-vine.aspx

Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: *Phytolacca americana* L.

Author(s) of the assessment:

- *Rob Tanner, European and Mediterranean Plant Protection Organization, Paris, France*
- *Guillaume Fried, ANSES, Montpellier, France*

Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: *Johan van Valkenburg, National Plant Protection Organization, Netherlands*
Peer review 2: *Giuseppe Brundu, University of Sassari, Sardinia, Italy*.

Date of completion: 17th September 2019

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: It should be noted that the Flora of North America (http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=220010427) detail that the "The infraspecific taxonomy of *Phytolacca americana* has been disputed since J. K. Small (1905) recognized *P. rigida* [*Phytolacca americana* var. *rigida* (Small) Caulkins & R.E. Wyatt, Bull. Torrey Bot. Club 117(4): 366 1990. basyonym: *Phytolacca rigida* Small, Bull. New York Bot. Gard. 3(11): 422–423 1905] as distinct from *P. americana* on the basis of its "permanently erect panicles" [sic] and "pedicels...much shorter than the diameter of the berries." J. W. Hardin (1964b) separated *P. rigida* from *P. americana* by the length of the raceme (2-12 cm in *P. rigida*, 5-30 cm in *P. americana*) and the thickness and diameter of the xylem center of the peduncle (70% greater thickness in *P. rigida*, 17% greater diameter in *P. americana*), but he found no discontinuities in any feature. J. W. Nowicke (1968) and J. D. Sauer (1952), among others, treated *P. rigida* as a synonym of *P. americana*. Most recently, D. B. Caulkins and R. Wyatt (1990) recognized *P. rigida* as a variety of *P. americana*."

Taxonomy:
Scientific name: *Phytolacca americana* L., Sp. Pl.: 441 (1753)

For this RA *P. americana* s.l. (in the broad sense) is considered especially in view of a lack of distinguishing character states and such a distinction not being made in Europe.

Kingdom: Plantae;
Phylum: Magnoliophyta;
Class: Angiospermae;
Order: Caryophyllales;
Family: Phytolaccaceae
Genus: *Phytolacca*

Synonyms:
Phytolacca americana var. *americana* L. (autonym)
Phytolacca decandra L., Sp. Pl. ed. 2: 631 (1762)

Note: Other checklist databases detail other synonyms for *P. americana* such as *Phytolacca vulgaris* Bubani, *Phytolacca vulgaris* Crantz (Bock *et al*., 2018).

English common names: American cancer, American nightshade, American pokeweed, American spinach, bears grape, cancer root, garget, inkberry, pigeonberry, poke, pokeberry, pokeroot, pokeweed, red-ink plant, stoke berry, Virginia poke.

Other languages: çapezë Albanian; американски лаконос, лаконос Bulgarian; američki kermes Croatian; líčidlo americké Czech; asiatisk kermesbær Danish; karmozijnbes, westerse karmozijnbes Dutch; laque, French: phytolacca américain, phytolaque américaine, phytolaque d'Amérique, phytolaque à dix étamines, raisin d'Amérique; amerikanische Kermesbeere, Scharlachbeere, Schminkbeere German; αγριοσταφίδα ή μαυροστάφυλο Greek; fitolakah amerikanit Hebrew; amerikai alkörmös Hungarian; cremesina uva-turca, erba carmesina, fitolacca, uva d'America, uva da colorare Italian; baga-noiva; erva-dos-cachos-de-índia, erva-dos-cancros, gaia-moça, tintureira, uva-da-américa, uva-dos-tintureiros, vermelhão, caruru-de-cacho, fruto-de-pombo, uva-de-tinta Portuguese; cîrmîz Romanian; лаконос американский Russian; америчка винобоја, гроздасти кермес Serbian; líčidlo americké; Slovakian; navadna barvilnica; Slovenian ; carmesín de oblea, espinacas de América, fitolaca, grana encarnada, granilla, hierba carmín, tintilla, uvas de América, uvas de Indiasombú Spanish; kermesbär Swedish; şekerciboyası Turkish; лаконос американський Ukrainian

Description of the species: *Phytolacca americana* is a polycarpic perennial herb (Armesto *et al.*, 1983) with a large white taproot which can reach 12 – 15 cm in diameter at ground level (Balogh & Juhász, 2008). The Flora of North America detail: 3 (-7) m in height. Leaves: petiole 1-6 cm; blade lanceolate to ovate, to 35 × 18 cm, base rounded to cordate, apex acuminate. Racemes open, proximal most pedicels sometimes bearing 2-few flowers, erect to drooping, 6-30 cm; peduncle to 15 cm; pedicel 3-13 mm. Flowers: sepals 5, white or greenish white to pinkish or purplish, ovate to suborbiculate, equal to subequal, 2.5-3.3 mm; stamens (9-)10(-12) in 1 whorl; carpels 6-12, connate at least in proximal $^1/2$; ovary 6-12-loculed. Berries purple-black, 6-11 mm diam. Seeds black, lenticular, 3 mm, shiny. $2n = 36$. Seeds can weigh 6.1 – 7.5 g/1000 seeds (Balogh & Juhász, 2008).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: In the horticultural trade within the risk assessment area plants traded as *Phytolacca rivinoides* and *Phytolacca latbenia* can be confused with. *P. americana* especially the morphological similarity and the colour of the inflorescence. In addition, both *Phytolacca acinosa* Roxb., *Phytolacca esculenta* Van Houtte and *Phytolacca polyandra* Batalin can be confused with *P. americana.*

Verloove (2019) detail that *P. acinosa* is an increasingly locally naturalised garden escape in Belgium. The species was first recorded in 1960 on a talus slope of the Albertkanaal in Kanne. From 1990 it has been recorded as an urban weed in many cities: Antwerpen, Brugge, Brussel, Gent, Izegem, Kortrijk, Leuven, Liège, Menen, Tielt, Tongeren occurring in gardens or parks in cemeteries or in urban wasteland. *P. polyandra* is locally naturalised in the British Isles (Clement & Foster 1994). *P. esculenta* is recorded as a casual alien plant in France (Tison & de Foucault, 2014). *P. acinosa* also occurs in Hungary as a non-native species (Balogh and Juhasz, 2008).

The risk assessment has not identified any native species within the risk assessment area where potential misidentification may occur with *P. americana.*

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: Following an extensive internet-based search, there are no risk assessments known for *Phytolacca americana* either within the RA area or at a global scale.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: *Phytolacca americana* is native to North America (including southeastern Canada, eastern the US and the northeast of Mexico) (Sauer, 1952; Rzedowski and Rzedowski, 2000). Its native range includes a little of southeastern Canada and almost the entire eastern half of the USA (Sauer, 1952). The species has however, spread westerwards into other States and USDA (2019) detail the species present in: Alabama, Arkansas, Arizona, California, Connecticut, District of Columbia, Deleware, Florida, Georgia, Iowa, Illinois, Indiana, Kansas, Kentucky, Louisiana, Massachusetts, Maryland, Maine, Michigan, Minnesota, Missouri, North Carolina, Nebraska, New Hampshire, New Jersey, New York, Ohii, Oklahoma, Pennsylvania, Rhode island, South Carolina, Tennessee, Texas, Virginia, Vermont, Wisconsin, and West Virginia (USDA, 2019; US NPGS, 2019). In Canada, the species is present in the Provinces: Brunswick, Ontario and Quebec. The spread of the species in North America is regarded as being greatly influenced by humans over the last few centuries (Sauer, 1952).

It is not possible for *P. americana* to naturally spread into the risk assessment area from its native range.

The species is native within a number of Kőppen-Geiger climate zones including, the main zones of Hot-summer humid continental climate (Dfa), Warm-summer humid continental climate (Dfb), humid subtropical climate (Cfa), Hot-summer Mediterranean climate (Csa).

Regarding the habitat preference of the species in its native range, it is often abundant in open, disturbed habitats, as well as in forest edges and light gaps (Sauer 1952, cited by Armesto et al (1983)). Balogh & Juhasz (2008) describe in more details that *"In its native range Ph. americana primarily grows as a pioneer plant of disturbed and open surfaces of damp soiled forests (for example around badger's burrows), on the fringe of forests and on riverbanks. Of the antropogeneous habitats it can be found on cuttings, waysides, fields and fallows. They prefer the eutrophic, flimsy, damp soils.*

It occurs rarely on sites where the temperature goes under –15 °C permanently in the winter, propagation is favourable if the average temperature is around 20 °C in July. In its native range it occurs at 1400 m of elevation."

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: *Phytolacca americana* has been introduced into many regions of the world. In Asia it is common from Turkey to Iran, and present in India, China, Taiwan, Japan, and Indonesia (on Sumatra it was found on 1500 m a.s.l.) (Balogh and Juhasz, 2008). The species is cultivated in China and recorded in the following provinces (Anhui, Fujian, Guangdong, Guizhou, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shandong, Sichuan, Taiwan, Yunnan, Zhejiang) (Flora of China, 2019). It has also been reported as invasive in China where it can become out of control in urban garden environments (Li *et al.*, 2016). The species is widely recorded in South Korea and considered an invasive alien species (Kim *et al.*, 2008). In Japan, the species is reportedly present throughout the country and it is estimated that the species was originally introduced into the country around 1970 (NIES, 2019). In Israel the species was first recorded in 1898 (Dufour-Dror 2012). In Turkey, the species is present around the Black Sea region from Samsun to Sarp/Artvin where invaded habitats included forests, river banks, waste land, coastal regions, urban and along the edges of agricultural areas (Akyol *et al.*, 2015).

In the Oceania region, the species is non-native in Australia, where it is found in New South Wales and Queensland (Hewson, 1984). It is also present in New Zealand (Webb *et al.*, 1988).

In Africa, *P. americana* is an invasive non-native species in South Africa (Invasive species South Africa, 2019), where it is listed as a NEMBA Category 1b species (i.e. "invasive species that may not be owned, imported into South Africa, grown, moved, sold, given as a gift or dumped in a waterway"). It is recorded as being problematic in Mpumalanga (Invasive species South Africa, 2019). Q bank (2019) also list the species as present in Cape Verde, Democratic People's Republic of Congo, Liberia, Mauritius, Reunion and Swaziland. Interestingly, the species is listed on the A1 list since 2001 for East Africa (EPPO, 2019).

Q bank (2019) lists central and South American countries where *P. americana* is present including: Costa Rica. Bolivia, Ecuador and Uruguay. There are some reports that the species is present in the Galapagos Islands (Charles Darwin Foundation, 2019).

The species is present in Switzerland where it is listed on the Observation list of Invasive Alien Plants since 2013 (EPPO, 2019). The species is reported to have been introduced into Switzerland in the 1700s as an ornamental plant (FOEN, 2006). It is mostly distributed south of the Alps, but some occurrences are recorded in northern Switzerland. It is reportedly a ruderal species growing in waste ground, disturbed habitats, open woods, pastures and along roadsides and railways (FOEN, 2006).

The species is present in Serbia where it has been recorded in woodland habitats in Vojvodina (Krtivojević *et al.*, 2012). It has been recorded in Ukraine (Balogh and Juhasz, 2008). The species is recorded as being non-native in Albania (Balogh and Juhasz, 2008).

The species is recorded as naturalised in Georgia with 107 recorded occurrences (Slodowicz *et al.*, 2018).

The species occurs as a non-native species in Macaronesia (Invasoras, 2019). It is present in the Azores archipelago (all islands) and the Madeira archipelago (Madeira island) (Invasoras, 2019).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).

Response (6a):

terrestrial biogeographic regions:

- Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic (based on GBIF data, 2019).

Response (6b):

terrestrial biogeographic regions:

- Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic (based on GBIF data, 2019).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic (see Figure 8, species modelling Annex VII).

Response (7b): Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic. With climate change there is the potential that areas of the alpine and boreal biogeographical regions may become suitable for the establishment of the species. Areas of the Mediterranean may become more limited for the establishment of the species (see Figure 8, species modelling Annex VII).

It should however be noted that the SDM may over represent the potential establishment of the species in the natural environment within the RA area as the data taken from GBIF to perform the models would also include localities where the species has been planted.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): Recorded: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Italy, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, United Kingdom

Response (8b): Established: Austria, Bulgaria, Croatia, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Italy, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain.

Further information on occurrence in EU Member States (where available):

Phytolacca americana was first recorded in Europe in the 17th century where its cultivation begun around the Mediterranean Sea (South Europe and North Africa), due to planting of the species as a dye-plant since 1650 (Balogh and Juhasz, 2008). From 1770 it started to spread out from Bordeaux (France). In Europe, the species has been introduced into Austria, Belgium (not established, Verloove, 2019), Bulgaria (Petrova *et al.*, 2013), Cyprus, Croatia (Mitíc *et al.*, 2006), Czech Republic, France (Le Neindre, 2002; Chabrol *et al.*, 2007), including Corsica (Jeanmonod *et al.*, 2011), Germany, Greece (Arianoutsou *et al.*, 2010) including Crete, Hungary (Botta-Dukát Z, Mihály B, 2006), Italy (Galasso *et al.*, 2008; Celesti-Grapow *et al.*, 2009) including Sardinia and Sicilia, Netherlands (J. van Valkenburg, pers comm. 2019), Portugal (Invasive Plants in Portugal, 2019, Romania, Slovenia, Slovakia, Sweden, Switzerland, Spain, UK (Stace, 2019). It should be noted, that in Belgium, there are increasing observations of the species, along with other *Phytolacca* species (e.g. *P. acinosa*) (Adriaens *et al*, 2019).

Borbás mentioned in 1879 that it started to escape around gardens and hedges in Budapest (Hungary). Domokos (1937) writes about it as a frequent plant in the Mecsekalja and along the Lower Danube already in the first half of the 20th century. Recent distribution in Hungary is South Transdanubia (mostly Belső-Somogy, West-Baranya), Duna-Tisza Interfluve (Budapest–Csévharaszt, to the south from Kecskemét) and Hajdúság (Téglás– Hajdúhadház). Recently its presence has been noticed in South-Mezőföld, but its smaller or bigger stands can be found in many other areas (for example eastern Vas County, Bakonyalja, Balaton-uplands, Gerecse, Külső-Somogy, Zselic).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): At present *P. americana* is established in Austria, Bulgaria, Croatia, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Italy, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain.It is envisaged that further establishment would be seen in these countries and other countries where the biogeographical regions are the same. Additional countries to the aforementioned would be Belgium, Estonia, Finland, Latvia, Lithuania and Luxembourg.

Response (9b): Under a climate change scenario of (RCP 4.5, over the next 30/50 years), countries in northern Europe may be suitable for the establishment of *P. americana* due to the increased temperature and the length of the growing season. Both increased summer and winter temperatures would benefit the species. Increased precipitation and CO2 levels as a result of climate change may also favor the species. These countries would include Denmark, Lithuania, Latvia, Estonia, Finland, Sweden and the UK. See Annex VII for more details.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: In the USA, in Pennsylvania, which is outside of its native range, *P. americana* is reported as a frequent weed in corn and soybean crops (Patches *et al.*, 2017; Steckel, 2006)). The species is also reported as a common pasture weed in the USA (Kiningham, nd). Sellers *et al* (2006) highlights that the species can be poisonous to animals and can impact on hogs, sheep, cattle, horses and poultry.

It has also been reported as invasive in China where it can become out of control in urban garden environments (Li *et al.*, 2016) In China, in natural reserves in Jiangsu, *P. americana* threatens the survival of native plants such as *Emilia sonchifolia* and *Taraxacum mongolicum* (Dong et al., 2011). The species is widely recorded in South Korea where it can invade coastal dune systems and has been designated as a harmful species because of its adverse effects on the ecosystem (Min 2014).

P. americana is an invasive non-native species in South Africa (Invasive species South Africa, 2019), where it is listed as a NEMBA Category 1b[2] species. It is recorded as being problematic in Mpumalanga where it spreads into natural habitats (Invasive species South Africa, 2019).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea,

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[2] **Category 1b**: Invasive species requiring compulsory control as part of an invasive species control programme. Remove and destroy. These plants are deemed to have such a high invasive potential that infestations can qualify to be placed under a government sponsored invasive species management programme.

Aegean-Levantine Sea

Response: Alpine, Atlantic, Continental, Mediterranean, Pannonian,

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response: France (Dumas, 2011), Germany (Schirmel, 2019), Hungary (Balogh and Juhasz, 2008), Italy (Acta plantarum, 2019), Poland (Chmura, 2016), Portugal (Invasoras, 2019), Romania (Szatmari, 2012), Spain (Sanz-Eloraza *et al*., 2001; Dana *et al*., 2001), Slovenia (Veenvliet *et al* 2017).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response: *Phytolacca americana* has numerous medicinal uses, these include achy muscles and joints (rheumatism); swelling of the nose, throat, and chest; tonsillitis; hoarse throat (laryngitis); swelling of lymph glands (adenitis); swollen and tender breasts (mastitis); mumps; skin infections including scabies, tinea, sycosis, ringworm, and acne; fluid retention (edema), skin cancers, menstrual cramps (dysmenorrhea), and syphilis. See Petit-Paly (1994), Patra et al 2014 for examples of chemical compounds.

Research is undertaken on the properties of natural compounds produced by *Phytolacca americana* e.g. Cho (2003), Getiya (2011).

The leaves of *P. americana* can be eaten – though they must be cooked, and apparently, it is used like spinach. The root is also reported as edible, though it is the most toxic part of the plant.

A dye can be obtained from the fruit, which can be used as ink and a dye for clothes (Balogh & Juhasz (2008). The ink can be used as body paint which American native Indians used. There are reports that the dye has been used as a food coloring and as a wine coloring agent.

It is reported that the roots are rich in saponins, which can be used as a substitute for soap.

Such an array of uses may be the reason why the species has expanded from its native range in the USA to cover most of the United States (Sauer, 1952).

Balogh & Juhasz (2008) detail that *P. americana* can also be used for the coloration of foods such as preserved fruit and sweets.

RHS reports the species being available in 16 nurseries in UK, see: https://www.rhs.org.uk/Plants/12895/i-Phytolacca-americana-i/Details

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided)”.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[3] and the provided key to pathways[4].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9

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[3] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[4] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Pathway name: **(1) Horticulture**

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	**CONFIDENCE**	high

Response: Entry via this pathway is deliberate, and planting of the species would be the end result of the movement of the species.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: *Phytolacca americana* is available through the horticultural trade both within the PRA area and outside. The species is available via internet suppliers (e.g. Amazon.com and ebay.com) but it remains unclear if the species can be sent to buyers within the EU from outside.

By definition, both seeds and whole plants could enter the RA area via plants for planting.

As entry via this pathway is deliberate, and planting of the species would be the end result of the movement of the species low numbers of propagules could result in the entry of the species.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: The pathway 'Horticulture' is the deliberate movement of plant material into the risk assessment area and as such plant material would be maintained and moved to ensure survival. No management practices would be carried out along this pathway.

It is unlikely that the species will reproduce or increase along the pathway. Both seed and live plants could be moved along this pathway.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: No management practices would be carried out along this pathway.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**unlikely**	**CONFIDENCE**	**high**

Response: It is unlikely that the organism will enter the risk assessment area undetected via the pathway ‘Horticulture (escape from confinement)’ is the deliberate movement of plant material into the risk assessment area.

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: *Phytolacca americana* is available through the horticultural trade both within the PRA area and outside. The species is available via internet suppliers (e.g. Amazon.com and ebay.com) but it remains unclear if the species can be sent to buyers within the EU from outside. Therefore, based on the latter, a medium rating of uncertainty has been given.

(2) Pathway name: **Transport – Contaminant (transport of habitat material (soil, vegetation))**

Qu. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: Entry via this pathway is unintentional movement of the species via the contamination of habitat material (soil and vegetation).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: The transport of topsoil and or other contaminated material with seed of the species can facilitate entry into the RA area. However, the pathway is mainly closed within the RA as there are prohibitions of the movement of soil into the EU from many countries.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: The pathway Transport – Contaminant (transport of habitat material (soil, vegetation) is the unintentional movement of plant material into the risk assessment area. As the seed would be moved with soil it is likely that they would survive during passage.

It is unlikely that the plant will multiply along the pathway

Seeds would be the most likely plant parts for transport, rather than whole plant parts. Seeds can remain dormant for a number of years and are thus likely to survive.

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Soil is unlikely to be treated as it is moved through the pathway and as such plant material would survive. Thus, a high rating of confidence has been given.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: It is likely that the organism will enter the risk assessment area undetected as seeds will be hidden in soil and may not be detected.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: The entry of *P. americana* via the pathway: Transport – Contaminant (transport of habitat material (soil, vegetation) has a moderately likelihood. The species can remain undetected within soil and other habitat material but the pathway remains closed for soil within EU countries (e.g. importation of soil and growing medium as such is prohibited in the EU, and is regulated when associated with plants (Regulation (EU) 2019/2072)).

(3) Pathway name: **Transport – stowaway (machinery/equipment)**

Qu. 1.2c. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: Entry via this pathway is unintentional movement of the species via machinery and equipment.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: Machinery and equipment used for forestry and agricultural purposes may include seeds of *P. americana* attached within tyre treads or other areas of machinery and equipment where soil is also attached.

There is no evidence that the species has entered the RA area via this pathway and there is no information available on the volumes of movement along this pathway. However, as in areas where the species is present, the seed bank density can be high, and thus there is a potential for seeds to become attached to tyres of vehicles. Dumas (2011), when considering spread, highlight that 'the transport of seeds by the soil retained in the tread pattern of machine tires is a 'hypothesis that we cannot exclude'. Thus, it should also be considered for movement into the RA area.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: The species is unlikely to reproduce along this pathway. But as the seeds are small the species can survive along the pathway.

Qu. 1.5c. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**unlikely**	**CONFIDENCE**	**low**

Response: If following cleaning methods suggested in ISPM 4, removing debris or filters - abrasive blasting - pressure washing - steam cleaning - sweeping and vacuuming - compressed air cleaning, potential survival of the species should be considered as low.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: Seeds are small and therefore they can remain undetected within crevices of used machinery.

Qu. 1.7c. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: Based on international Standards and requirements within (ISPM 41: FAO, 2017), the entry of *P. americana* as a hitchhiker of used machinery is moderately likely. However, incorrect application of the cleaning of machinery could lead to the entry of seed via this pathway. In addition, volumes of movement of used machinery into the RA area is not known and therefore the uncertainty is scored low, in part to reflect this.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: When considering all pathways into the RA area, it is likely that *P. americana* can enter the RA area with a medium confidence. All biogeographical regions would have similar likelihood scores based on the pathways described.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Within the next 30/50 years, under the medium climate change prediction (RCP 5.5), there is an overall likely score that *P. americana* will enter the RA area with a medium confidence. As this question is only considering introduction into the RA area, all biogeographical regions would have similar likelihood scores based on the pathways described. Climate change is unlikely to change the current pathways but it may extend the areas where the species can be grown to the north and restrict the areas where the species may grow in the Mediterranean region, i.e. regions where someone could introduce it for planting could change accordingly

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[5] and the provided key to pathways[6].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name: **(1) Horticulture (escape from confinement).**

Qu. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	**medium**

Response: The pathway is the escape of the species from horticulture into the natural environment. Thus, this in its strictest definition would be an unintentional occurrence of the species in the environment outside of cultivation.

RHS reports the species being available in 16 nurseries in UK, see: https://www.rhs.org.uk/Plants/12895/i-Phytolacca-americana-i/Details

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[5] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[6] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: There is no information on the volumes of movement along this entry pathway. A single plant can produce over 2 500 fruits and 25 000 seeds (Dumas 2011).

These fruit can be eaten by birds and seeds transferred from the confines of a garden into the wild of the RA area (Li et al., 2016). In addition, the species can grow to reasonable heights and potentially overhang garden fences and walls where it can release fruit into the natural environment.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Fruit/seeds can enter the risk assessment area from garden sources and can remain undetected until they germinate and grow. Seeds can remain viable in the seed bank for a long period of time (DiTomaso et al., 2013).

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Seeds would enter the environment during summer and autumn months and the seeds be included in the soil and remain viable in a seed bank.

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: As previously mentioned, the fruit/seeds can be moved via birds and other entry pathways including small mammals and also via dropping seeds over walls or garden fences from plants contained within gardens.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**very likely**	**CONFIDENCE**	**medium**

Response: It is very likely that the species can enter the environment within the risk assessment area based on the pathway Horticulture (escape from confinement).

Pathway name: **(2) Release in nature for use**

Qu. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional	**CONFIDENCE**	**high**

Response: *Phytolacca americana* can be used for a number of purposes, especially medical purposes including use for achy muscles and joints (rheumatism); swelling of the nose, throat, and chest; tonsillitis; hoarse throat (laryngitis); swelling of lymph glands (adenitis); swollen and tender breasts (mastitis); mumps; skin infections including scabies, tinea, sycosis, ringworm, and acne; fluid retention (edema), skin cancers, menstrual cramps (dysmenorrhea), and syphilis.

In addition, the species can be used as a food plant.

This entry pathway deals with uses of the species other than horticulture and would involve the deliberate planting of the species.

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment

along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**unlikely**	**CONFIDENCE**	**low**

Response: The species would be deliberately planted within the environment. It is not expected that large numbers would enter the environment via this pathway mainly as the species is only likely to be used as a medical or food plant by a very limited number of the population within EU Member States.

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**very unlikely**	**CONFIDENCE**	**high**

Response: The species would be deliberately planted within the environment.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: The species would be deliberately planted within the environment. Seeds would most likely be planted during the spring and summer months and the seeds be included in the soil and remain viable in a seed bank.

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: The species would be deliberately planted within the environment and it is very likely that those people who plant the species would be planting it in habitats that are suitable for its growth.

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: It is moderately likely that the species can enter the environment within the risk assessment area based on the pathway release in nature for use. The overall score is lower than that for horticulture escape from confinement as the use of the species for medicinal purposes or as a food plant would be considerably lower than that for horticulture use.

Pathway name: **(3) Transport – Contaminant (transport of habitat material (soil, vegetation)**

Qu. 2.2c. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	**medium**

Response: The pathway is the entry of the species as a contaminant of habitat material (soil or vegetation) into the natural environment. Thus, this is the unintentional occurrence of the species in the environment.

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: There is no information on the volumes of movement along this entry pathway. As the pathway is mainly closed within EU Member States, the likelihood is only moderately likely with a low uncertainty.

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Seeds can enter the environment as a contaminant undetected as they are small and may be hidden in habitat material.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: Such material could enter the environment at any time of the year and seeds are long lived and can remain viable for a number of years.

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**very likely**	**CONFIDENCE**	**medium**

Response: The material relevant to this pathway is habitat material (soil or vegetation) and as such this material could be deliberately placed in a suitable habitat within the environment where the seed contaminants could enter the environment.

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: It is moderately likely that the species can enter the environment within the risk assessment area based on this pathway.

Pathway name: **(4) Transport – stowaway (machinery/equipment)**

Qu. 2.2d. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	**unintentional**	**CONFIDENCE**	**medium**

Response: The pathway is the entry of the species as a stowaway of machinery/equipment into the natural environment. Thus, this is the unintentional occurrence of the species in the environment.

Qu. 2.3d. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: There is no information on the volumes of movement along this entry pathway. There is also no information on the volumes of movement of used machinery/equipment from outside the RA area into the RA area. As detailed in the response to Qu. 1.3.c, movement along this pathway has been considered for spread and it is also possible for the movement from non-EU countries bordering EU countries.

Qu. 2.4d. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Seeds are small and can remain undetected within crevices of used machinery. Without proper cleaning of equipment at source, or before it enters the environment, it is likely that seed can remain as a contaminant of used machinery

Qu. 2.5d. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: Used equipment, especially equipment used for forestry management etc. could enter the environment at any time of the year and seeds are long lived and can remain viable for a number of years.

Qu. 2.6d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Used equipment, especially equipment used for forestry management etc. could be deliberately placed in a suitable habitat within the environment where the seed hitchhikers could enter the environment.

Qu. 2.7d. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: It is moderately likely that the species can enter the environment within the risk assessment area based on this pathway.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	**likely**	**CONFIDENCE**	**High**

Response: When considering all pathways into the RA area, it is likely that *P. americana* can enter the environment within the RA area with a high confidence. All biogeographical regions would have similar likelihood scores based on the pathways described.

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: When considering all pathways into the RA area, it is likely that *P. americana* can enter habitats within the RA area with a high confidence. Climate change may extend the areas where the species can be grown to the north and restrict the areas where the species may grow in the Mediterranean region. Both increased summer and winter temperatures would benefit the species. Increased precipitation and CO2 levels as a result of climate change may also favor the species. All biogeographical regions would have similar likelihood scores based on the pathways described.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: *Phytolacca americana* is already established within the RA area (Austria, Bulgaria, Croatia, Cyprus, Czech Republic, France, Germany, Greece, Hungary, Italy, Netherlands, Malta, Poland, Portugal, Romania, Slovakia, Slovenia, Spain). It is likely that further areas of establishment are present within the RA area.

In its native range, the species is established in Kőppen-Geiger climate zones of Dfa, Dfb, Cfa, Csa. All of these Kőppen-Geiger climate zones are present within the EU and the habitats where the species can persist are present throughout the RA area.

Species modelling shows that *P. americana* has the potential to establish over much of the European Union, see Annex VII.

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	**widespread**	**CONFIDENCE**	**high**

Response: Balogh and Juhasz (2008) detail that *P. americana* is mostly a ruderal species growing in disturbed habitats. The species is able to grow in both sunny and shady sites. In its native range, *P. americana* primarily grows as a pioneer plant of disturbed and open surfaces of damp soiled forests (for example around badger's burrows), on the fringe of forests and on riverbanks. Of the anthropogeneous habitats it can be found in cuttings, waysides, fields and fallows. The species prefer the eutrophic, flimsy, damp soils. It occurs rarely on sites where the temperature goes under –15 °C permanently in the winter, propagation is favourable if the average temperature is around 20 °C in July. In its native range it occurs up to 1400 m of elevation.

Within the Europe, the species occurs in clear-cut areas (for example in Austria, Lajta hills), and along hedgerows and wasteland (e.g. in Switzerland). Balogh and Juhasz (2008) report that in Italy it can be found on field sides, along canals, along the coast, and in black locust plantations. The species is found in forest plantations in Hungary and in disturbed woodlands. In addition, in Hungary, the species is found in sandy grassland and alder swamp forests that has no surface water. It prefers the more humid habitats, and the half-shade; on sunny sites it grows usually under shrubs or trees. Balogh and Juhasz (2008) highlight that the species generally favours loose soils that developed on acidic or neutral, sandy or pebble bedrock. Dumas (2011) detail that the species can also grow on limestone, the edges of streams. In France, the species can be found in riparian habitats, clearings and forest edges, near dwellings, in wastelands, railway stations, old quarries, rubble, and corn crops (Fried, 2017). It prefers sandy and / or humus soils.

In the EU, the species can be found growing in uncultivated vineyards, orchards, and arable fields and row crop cultures (paprika, tomato, sunflower) (Balogh and Juhasz,2008). The species is often found growing within urban areas where it can form dense stands if the land is left unmanaged.

The species has been recorded in natural habitats, in particular in Carei Plain Natural protected area in Western Romania where it is reported to occur in natural and planted forests and anthropogenically affected areas (Szatmari, 2012).

All of the aforementioned habitats are widespread within the RA area.

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	**N/A**	**CONFIDENCE**	**high**

Response: *Phytolacca americana* does not require another species for any critical stage in its lifecycle.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: It is very likely that *P. americana* will establishment despite competition from existing species. *P. americana* is highly competitive species which has been shown to successfully outcompete native plant species within the RA area. The ability of the species to form dense monocultures, coupled with the lack of natural enemies provides the species with an advantage over native species.

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	**very likely**	**CONFIDENCE**		**high**

Response: *Phytolacca americana* is native to North America and thus co-evolved natural enemies would be present in this region and not within the risk assessment area. Those more generalist organisms naturally present in the risk assessment area, which might feed on the species, are unlikely to prevent the establishment of the species.

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: There are a number of management practices applied to *P. americana* within the risk assessment area. However, these management practices are mainly applied to established populations or where populations may start to establish in areas of high conservation value. Other areas, such as ruderal habitats may be overlooked and therefore provide habitats for establishment despite existing management practices.

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: As detailed in section (3.2), the establishment of *P. americana* is suited to disturbed habitats especially disused waste ground. It is therefore likely that the current urbanization trend occurring in Europe may favor the establishment of the species. Additionally, management practices in forests, may act to open the canopy and favour disturbance that would be beneficial for the germination of the seedbank.

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: The species produces a large amount of seed, which can remain dormant for long periods of time. It also has a high seed bank density and thus all seeds would need to be removed. Therefore, these factors may hinder eradication efforts. In addition, the seed bank may be widespread as the plant can be widely spread by birds and other animals.

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union

- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: *Phytolacca americana* can reproduce by both seed and regeneration from underground rhizomes. Each inflorescence can contain numerous berries, each containing 10 seeds. McDonnell *et al.* (1984) counted up to 78 ripe fruits per inflorescence. A single plant can produce over 2 500 fruits and 25 000 seeds (Dumas 2011). Dumas (2011), citing Armesto (1983) estimate a density of 592 seeds per m-1. The species is self-fertilizing and flowering can occur in the first year of growth. Mature berries can occur from August to early November (France) and germination rates have been reported as high as 80 % though it varies within the population (0 – 100 %) (Armesto *et al.*, 1983; Vuillemenot & Mischler, 2012). Seeds can remain viable in the soil for approximately forty years (Vuillemenot & Mischler, 2012) and can germinate following disturbance in the soil and/or a clearing of an area (for example a woodland). This has been shown to be the case in the ‘Massif de la Serre’ forest in the Jura, France (Vuillemenot & Mischler, 2012).

It is interesting to note that germination of seed is increased when the seed moves through a bird digestive tract (Dumas, 2011). It is noted that, ‘Orrock (2005) studied the influence that can have the transit of seeds in the digestive tract of birds where a positive effect on the germination rate, which goes from 67% for controls to 88% for seeds from the fruits consumed’.

In addition, the species appears to be resistant to a number of environmental constraints. For example, the species can withstand high levels of heavy metals in soils enabling the species to grow in polluted habitats (Min *et al.* 2006; Peng, 2008). It is noted that soil texture and acidity does not limit the occurrence of *P. americana* (Sauer, 1952). The species is reported to tolerate a wide range of soil pH (Sauer, 1952).

Seed of the species appears to be tolerant of fire and can promote its germination. After forest fires, seed can germinate from the seed bank (Glasgow *et al.*, 2007).

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: *Phytolacca americana* has a high level of plasticity being tolerant to a variety of environmental conditions and as such habitats. The species thrives in ruderal habitats where disturbances occur (Dumas, 2011).

There are some indications that the species has adapted to the RA area and as detailed by Dumas (2006), the climatic limits of the species in the RA area are unlikely to have been reached. Dumas

(2011) highlight that if the climatic characteristics defined by Sauer (1952) in the area of origin, are applied to France, the species should not be present in the Fontainebleau region, where it is currently widespread. This region of France does not meet the temperature and rainfall requirements.

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: The species has established within the RA area though it is not known how many introductions have taken place from founder populations to realize this establishment.

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: The species is already established within the RA area. In areas in the RA area where it is not established, as the species is spread by birds and other animals, casual population of the species may occur with the RA area in space and time.

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: *Phytolacca americana* is present in the Koppen Giger climate zones of Csb, Csc, Cfb within the EU. Csb (warm-summer Mediterranean climate) and Cfb (oceanic climate with warm summers) are widespread zones within the EU.

Phytolacca americana is present in the following biogeographical regions: Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic (based on GBIF data, 2019).

The species still has the potential for further establishment in the aforementioned biogeographical regions.

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Under a climate change scenario of (RCP 4.5, over the next 30/50 years), when considering establishment into the RA area, it is likely that *P. americana* will establish within the RA area with a medium confidence. Climate change may extend the areas where the species can be grown to the north and restrict the areas where the species may grow in the Mediterranean region (see species distribution modelling annex). Both increased summer and winter temperatures would benefit the species. Increased precipitation and CO2 levels as a result of climate change may also favor the species. An increase in fires within the RA area due to increased temperature may act to promote the germination of the seed bank and increase the population. All biogeographical regions would have similar likelihood scores based on the pathways described. See Annex VII for more details.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic “spread” via release or escape (“jump-dispersal”), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	**major**	**CONFIDENCE**	**medium**

Response: Natural spread is a key factor in the dispersal of *P. americana* in the RA area. Birds have been reported to spread the species within the RA area. In America, it is reported that up to 29 bird species feed on the fruits of *P. americana* (Armesto, 1983). Within the RA area, some data exist. For example, Balogh and Juhasz (2008) detail the following species feeding on fruits in the following countries: in Italy: blackcap (*Sylvia atricapilla*), whitethroat (*Sylvia communis*), song trush (*Turdus philomelos*), blackbird (*Turdus merula*), blue rock trush (*Monticola solitarius*), robin (*Erithacus rubecula*); in South France: robin and blackcap; in New-Zeland: the pheasant (*Phasianus colchicus*). Villemenot & Mischler (2012) list several bird species eating fleshy berries as responsible of *P. americana* spread: pigeons (*Columba* spp.), turtledoves (*Streptopelia decaocto*) and starling (*Sturnus vulgaris*), but also probably blackbirds (*Turdus merula*), thrushes (*Turdus* spp.) and warblers (*Sylvia* spp.).

Sauer (1952) report that the species is poorly suited to dispersal by wind or water (information from the native range, Sauer, 1952).

Benvenuti (2004) detail that the starling (*Sturnus vulgaris*), is the main species responsible of this spread in the city of Pisa, *P. americana* grows under trees the species chooses as nesting sites. He also notes that the latter germinate faster (4 to 5 days earlier) than the control seeds.

Balogh and Juhasz (2008) write: ‘Ad hoc observations prove that the young shoots of *P. americana* is eaten by big games (red deer, fallow deer) living in the Hungarian forests. The sheep and the goat kept on sandy pasture-land consume this plant too'. However, it is not known if they can act to spread the plant through seed. In addition, Dumas (2011) include rodents as seed feeders. In the forest of Fontainebleau, cervids are also suspected to be vectors of the seeds (Villemenot & Mischler, 2012). Indeed, all such species have the potential to spread seed within the RA area.

It should be noted, that in Belgium, there are increasing observations of the species, along with other *Phytolacca* species (e.g. *P. acinosa*) (Adriaens *et al*, 2019).

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Response: Human assisted spread can play a factor in the spread of the species due to the use of machinery and management of certain habitats (such as woodland management). Vuillemenot & Mischler (2012), detail that harvesters in softwood plantations have acted to promote the spread of the species. The intervention of harvesters in the softwood plantations of would trigger the germination of the seed bank, due to ground disturbances.

Vuillemenot & Mischler (2012) also highlight that seeds can become incorporated into the tread of tyres and hiking boots that can then act to spread the species into new areas.

Soil can also contain large amounts of seeds (and it is detailed that the seed bank can have a longevity of up to 40 years), thus the movement of soil may also act to facilitate the spread of the species within the RA area.

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after

eradication.

- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name: Transport – stowaway: Machinery/ equipment

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: The spread of *P. americana* via machinery/ equipment is the unintentional spread of the species within the RA area.

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: There is no information available on the volumes of movement along this pathway. However, as in areas where the species is present, the seed bank density can be high, and thus there is a potential for spread. Dumas (2011), when considering spread, highlight that ‘the transport of seeds by the soil retained in the tread pattern of machine tires is a hypothesis that we cannot exclude’.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: The seeds of the species are small and therefore they could survive during transport along this spread pathway. The species is unlikely to increase along the pathway until it finds a suitable habitat.

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: With the right cleaning and disinfecting of used machinery, the species would probably be removed from the machinery. However, such practices are not always common and therefore the species may survive existing management practices during this mode of spread.

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: The seeds are relatively small and potentially can remain hidden in soil in cracks and crevices in machinery and equipment. Such machinery can be moved around the RA area transporting the seed to new areas.

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: It would be very likely that *P. americana* can transfer to a suitable habitat if the species is a stowaway on machinery/ equipment. The machinery in question, such as forest vehicles or harvesters are utilized in suitable habitats.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately**	**CONFIDENCE**	**low**

Response: The overall potential rate of spread within the RA area for the pathway Transport – stowaway: Machinery/ equipment is rated as moderately with a low confidence. Dumas (2011), has highlight this pathway as a potential pathway, but as previously mentioned, there is no information on rates of spread, hence the low confidence score.

Pathway name: UNAIDED (natural spread)

Qu. 4.3b. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: The spread of *P. americana* via natural methods (birds and other small mammals) is the unintentional spread of the species within the RA area. As highlighted in question 4.1, natural spread is considered a significant pathway for spread of the species in the RA area.

Qu. 4.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: There is no information available on the volumes of movement along this pathway. Natural spread is a key factor in the dispersal of *P. americana* in the RA area. In particular, birds have been reported to spread the species with the RA area. In America, it is reported that up to 29 bird species feed on the fruits of P. americana (Armesto, 1983). Within the RA area, some data exist. For example, Balogh and Juhasz (2008) detail the following species feeding on fruits in the following countries: in Italy: blackcap (*Sylvia atricapilla*), whitethroat (*Sylvia communis*), song trush (*Turdus philomelos*), blackbird (*Turdus merula*), blue rock trush (*Monticola solitarius*), robin (*Erithacus rubecula*); in South France: robin and blackcap; in New-Zeland: the pheasant (*Phasianus colchicus*).

Sauer (1952) report that the species is poorly suited to dispersal by wind or water (information from the native range, Sauer, 1952).

Benvenuti (2004) detail that the starling (*Sturnus vulgaris*), is the main species responsible of this spread in the city of Pisa, *P. americana* grows under trees the species chooses as nesting sites. He also notes that the latter germinate faster (4 to 5 days earlier) than the control seeds.

Balogh and Juhasz (2008) write: ‘Ad hoc observations prove that the young shoots of *P.. americana* is eaten by big games (red deer, fallow deer) living in the Hungarian forests. The sheep and the goat kept on sandy pasture-land consume this plant too'. In addition, Dumas (2011) include rodents as seed feeders. Indeed, all such species have the potential to spread seed within the RA area.

Qu. 4.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: The seeds of the species can remain viable following movement through the digestive system of animals and birds. Thus, seeds can survive, though they will not reproduce or increase during this spread pathway.

Qu. 4.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	**very likely**	**CONFIDENCE**	**medium**

Response: Management practices will be very limited for this spread pathway. Controlling birds and small mammal movement is not an option for management.

Qu. 4.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Birds and other small mammals can carry the seeds over large distances and can be dispersed in areas undetected. The seeds will remain in the gut of the species for some time and be deposited in the soil.

Qu. 4.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**very likely**	**CONFIDENCE**	**high**

Response: Birds and other small mammals can carry the seeds over large distances and the seeds can be dispersed in habitats suitable for the species.

Qu. 4.9b. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**rapidly**	**CONFIDENCE**	**medium**

Response: The overall potential rate of spread via this pathway is estimated as rapidly with a medium confidence.

Pathway name: Transport – contaminant (transport of habitat material (soil, vegetation)

Qu. 4.3c. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: The spread of *P. americana* via transport of habitat material (soil, vegetation) is the unintentional spread of the species within the RA area.

Qu. 4.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: There is no information available on the volumes of movement along this pathway. However, as in areas where the species is present, the seed bank density can be high, and thus there is a potential for spread.

Qu. 4.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: The seeds of the species are small and therefore they could survive during transport along this spread pathway. The substrate would be conducive to maintain survival.

Qu. 4.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: Careful methodical management practices coupled with inspection would be needed to ensure that the species did not spread with contaminated soil. This is often not feasible with such small seeds.

Qu. 4.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: The seeds are relatively small and potentially can remain undetected within soil. Soil and other habitat material can be moved throughout the RA area and seeds can be spread within such material.

Qu. 4.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: It would be very likely that *P. americana* can transfer to a suitable habitat if seed of the species is incorporated in soil. Topsoil and habitat material is often physically transferred to suitable habitats and thus it is very likely that the species will transfer to suitable habitats.

This fact that the species is often recorded in urban development areas further supports the hypothesis that the species can be moved by soil and habitat material.

Qu. 4.9c. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately**	**CONFIDENCE**	**medium**

Response: Response: The overall potential rate of spread via this pathway is estimated as moderately with a medium confidence.

Pathway name: People and their luggage/ equipment (in particular tourism)

Qu. 4.3d. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: The spread of *P. americana* via people and their luggage/equipment in particular tourism is for this species considered the unintentional movement of the species via seeds. In the sense of this pathway, the main risk is of introduction into new areas via spread is that the species could be incorporated into the tread of hiking boots or other equipment and moved accidently to other areas.

Qu. 4.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	**moderately likely**	**CONFIDENCE**	**low**

Response: There is no information available on the volumes of movement along this pathway. As the fruit of the species does not have any spiny spurs there would be less chance of the fruits attaching to clothes and other material. This spread pathway is not considered by the authors of this RA as a major spread pathway.

Qu. 4.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	**moderately likely**	**CONFIDENCE**	**medium**

Response: The seeds of the species are small and therefore they could survive during transport along this spread pathway. The substrate would be conducive to maintain survival. The species would not reproduce along this pathway or increase.

Qu. 4.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	**likely**	**CONFIDENCE**	**medium**

Response: Careful methodical management practices coupled with inspection of tread on boots would be needed to ensure that the species did not spread. However, often biosecurity measures are not widely known by the general public.

Qu. 4.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: The seeds are relatively small and potentially can remain undetected within the tread of hiking boots. Additionally, the seeds may not be easily identifiable to non-botanists and thus may be overlooked.

Qu. 4.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	**likely**	**CONFIDENCE**	**high**

Response: It would be very likely that *P. americana* can transfer to a suitable habitat if seed of the species is attached to hiking boots. Top soil and habitat material is often physically transferred to suitable habitats and thus it is very likely that the species will transfer to suitable habitats.

This fact that the species is often recorded in urban development areas further supports the hypothesis that the species can be moved by soil and habitat material.

Qu. 4.9d. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	**moderately**	**CONFIDENCE**	**medium**

Response: Response: The overall potential rate of spread via this pathway is estimated as moderately with a medium confidence.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	**difficult**	**CONFIDENCE**	**high**

Response: The spread pathways are numerous and varied making the management of the spread pathways difficult. In particular, natural spread is a difficult pathway to manage.

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	**rapidly**	**CONFIDENCE**	**medium**

Response: The species is currently present in the following biogeographical regions: Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic (based on GBIF data, 2019). Within all of these regions, the spread is likely to be rapidly.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	**rapidly**	**CONFIDENCE**	**medium**

Response: Climatic change will allow *P. americana* to establish further north than present but its inherent rate of spread should remain rapidly with a medium confidence.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comment: *Phytolacca americana* is reported to form dense stands that can outcompete native vegetation and can act to retard forest regeneration (FOEN, 2006; Orwig *et al.*, 1998). Apart from this and other references to the species being invasive (e.g. in China, Dong et al., 2011), there are no other known studies that have evaluated the impact of the species on biodiversity.

However, as the species is able to form dense stands these may outcompete native plant species for space, light and nutrients.

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comment: Dumas (2007) indicates that as soon as *P. americana* reach 50% cover, there is 24% decrease in species richness of invaded communities. When it is abundant, *P. americana* can modify several plant resident plant communities, by competing notably *Rubus* spp. and *Cytisus scoparius*, especially in all the open environments associated with forest, such as the hems and the clearings, megaphorbiaies, wastelands and moors, etc. (Villemenot & Mischler, 2012).

However, Dumas (2011) considers that studies on the impact of *P. americana* on native biodiversity within the RA area is poorly documented. In addition, Fried (2012) considers that overall the impact of this species is low, since the vast majority of *P. americana* populations are found in ruderal or post-crop areas (vines abandoned).

A study by Schirmel (2019) in southwest Germany, showed that *P. americana* invasion resulted in an altered arthropod community structure. The cricket *Nemobius sylvestris* was negatively affected by *P. Americana*.

There are suggestions, that chemical leaching from seeds fallen to the ground may also be toxic to the soil macro- and micro-biota (Dumas, 2011).

Balogh and Juhasz (2008) detail that *P. americana* can out-compete native species on sandy grasslands by completing for space and light. The species can shade out native species and in different forest communities its presence can reduce the conservation value. Dispersion of *P. americana* in the protected area of Barcsi Borokas (originally with dominance of *Juniperus communis*) causes large problems, where along with an invasive tree, black cherry (*Prunus serotina*) it occurs in mass in open perennial grasslands (*Festuco-Corynephoretum*), Molinia-Turkey oak forests (*Molinio litoralis-Quercetum*) and alder swamp forests (*Carici elongatae-Alnetum*) too. In West Hungary it also endangers the oak-hornbeam forests.

Campana *et al.* (2002) detail a disruptive impact on earthworm populations highlighting that the species seem to repel most earthworm species. This may be due to the allelopathic properties of the species. Given its molluscicidal potency, *P. americana* probably has the same effect on gastropods (Villemenot & Mischler, 2012).

Henneuse *et al.* (2007) observed a reduction in plant species richness when the recovery of *P. americana* increases.

Phytolacca americana has been detailed as one of the top invasive plants (most harmful) by Protected Area Managers where it was highlighted as present in 4 Protected Areas (Monaco and Genovesi, 2014). In comparison, *Fallopia japonica* was highlighted in 48 Protected Areas

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comment: Impacts, although not currently scientifically evaluated, are likely to be moderate in the future as the species can form dense monocultures which can outcompete native plant species. Further spread is likely throughout the RA area, especially in ruderal habitats and forests.

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comment: There are no detailed scientific studies to evaluate the impact of *P. americana* on native species and thus any decline in conservation value to habitats. As mentioned, the species predominantly grows in ruderal habitats within the RA area and these are often of little conservation importance. However, in the areas where the species is established, *P. americana* has invaded natural habitats which can act to decrease local biodiversity (see question 5.2).

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comment: There are no detailed scientific studies to evaluate the impact of *P. americana* on native species and thus any decline in conservation value to habitats. As mentioned, the species predominantly grows in ruderal habitats within the RA area and these are often of little conservation importance. However, in the areas where the species is established, *P. americana* has invaded natural habitats which can act to decrease local biodiversity (see question 5.2).

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	**moderate**	**CONFIDENCE**	**low**

There are no scientific studies that have evaluated the impact of *P. americana* on ecosystem services in the RA area. There is some anecdotal evidence that the species can retard forest regeneration (Dong et al., 2011) though further research is needed on the subject. According to Dumas (2011), another effect of *P. americana* on abiotic conditions would be the enrichment of potassium that this species causes on soils, constituting reserves of this element in the biotope.

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comment: Vuillemenot & Mischler (2012) detail that the description of the species to natural forest regeneration is often mentioned as a negative impact. The presence of the species may affect some recreational activities especially if the species forms dense monocultures in natural habitats blocking pathways and other recreational areas. The dark pigment of the fruit may also stain clothes.

The species has been reported as having allelopathic properties which can affect the microbial soil community other organisms though there has not been any research conducted on this aspect at present.

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural

services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comment: *Phytolacca americana* may spread northwards within the RA area as a result of climate change though the impacts score is likely to be the same as under the current situation (moderate with a medium confidence). Impacts in the Mediterranean may be less if the climate is not conducive to establishment.

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**minor**	**CONFIDENCE**	**low**

Comment: *Phytolacca americana* can incur control costs within the risk assessment area particularly in clearing the species from areas where it has colonized urban development sites. In addition, the species can impact on woodland plantation as the species will need to be cleared and eradicated prior to planting of forest trees.

In the USA, there are reports the species can impact on yields of various crops though there are no direct studies that have evaluated crop yield reduction due to the presence of *P. americana*. In Pennsylvania, studies have been conducted to assess chemical control options for the species in maize and soybean fields highlighting there is management of the species and a cost associated (Patches et al., 2017).

Steckel (2006) highlights that the stain (from the berries) can impact on soybeans during harvest.

Although no monetary figure exist on costs associated with *P. americana* control or damage, costs are likely as Steckel (2006) highlights that the species can be very competitive for row crops. However, it is likely that the species is a minor economic problem compared to other weedy species in North America (e.g. Amaranthus palmeri – where there are numerous publications highlighting 70 -80 % crop yield reductions).

Cucumber mosaic virus (CMV) has been reported in *P. americana* plants from northern Italy (Davino et al., 2012). This could have economic impacts to plant health within the EU.

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found". Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comments: The species has been shown to be problematic in maize crops, especially due to the berries that can release their liquid when crushed and stain the crops. Steckel (2006) highlights that the stain can impact on soybeans during harvest.

It is locally an important weed in maize crops of southwestern France where its harmfulness is considered very high (Mamarot & Rodriguez, 2002). It is also a weed in forestry where it can damage young plantations of trees (Villemenot & Mischler, 2012). In forest patches driven by natural regeneration, such as a mature oak forest whose coppice has been cut, the invasion of *P. americana* seems much more important. In this case, the density of *P. americana* stands questions the possibility of germination and development of young trees (Villemenot & Mischler, 2012). It has also been supposed that the reduced dietary interest for cervids of plots largely invaded by *P. americana* can reduce the hunting interest of some forests and the related income for forest owners (Villemenot & Mischler, 2012).

Kumschick *et al*. (2015) score the species as 1 for socio-economic costs 'Minor impacts, in the range of native species, only locally, negligible economic loss'.

Cucumber mosaic virus (CMV) has been reported in *P. americana* plants from northern Italy (Davino et al., 2012). This could have economic impacts to plant health within the EU.

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comments: It is locally an important weed in maize crops of southwestern France where its harmfulness is considered very high (Mamarot & Rodriguez, 2002). It is also a weed in forestry where it can compete for light and resources with young plantations of trees (Villemenot & Mischler, 2002). In plots that have been cleared and where young tree seedlings have been introduced, the presence of *P. americana* can be an inconvenience to the forester, by requiring more regular clearing work until the tops of the young planted trees exceed the *P. americana* stands. Once this stage is over, the young trees released from competition will grow; but even if the density of the *P. americana* declines due to the shade created by the new settlement, this species seems to be maintained apparently for a long time and compete with species in shrub and herbaceous strata.In forest patches driven by natural regeneration, such as a mature oak forest whose coppice has been cut, the invasion of *P. americana* seems much more important. In this case, the density of *P. americana* stands questions the possibility of germination and development of young trees (Villemenot & Mischler, 2002). With an increase in geographical occurrence, the species may potentially cause greater economic costs.

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	**moderate**	**CONFIDENCE**	**low**

Comments: The weeding of this species is costly in maize crop where herbicide spraying is only efficient on seedlings, but not on regrowth, and it requires an additional spray increasing the weed management cost (Mamarot & Rodriguez, 2003). Villemenot & Mischler (2012) also indicate that in forest, *P. americana* requires more regular clearance work until the top of the young trees exceeds *P. americana*. However, as the species often invades ruderal habitats and waste land, the economic management cost of the species is likely to be moderate within the RA area.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comments: No information has been found on the issue. With an increase in geographical occurrence, the species may potentially cause greater economic costs but this is difficult to predict.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of

people, property or infrastructure;

- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comments: *Phytolacca americana*, although edible, is a toxic species that cause vomiting and diarrhea when eaten raw. Balogh and Juhasz (2008) detail: 'The most toxic part of the plant is the root, which contains saponins, among them phytolaccatoxin that is toxic to vertebrates. In respects of human health the most dangerous is the lectin content. In the new roots of *P. americana* hemagglutinin compound was detected – which is similar to the ones in the seeds of castor bean and Calabar bean – that contains much cysteine (a sulphur-laden amino-acid) and has mitogenic effect. It can stimulate the abnormal cell division of the poise B- and T- limphocytes, and it can damage the chromosomes too'.

Ogzewalla *et al.* (1962) highlights that in the USA, children can often eat the berries and become ill. The fruits, due to their deep red colour can be inviting to children and can resemble berries of other species (similar to that in the RA region). There are reports of deaths through consuming *P. americana*, though it is generally reported that such fatalities are uncommon.

Phytolacca americana can also be toxic to animal species. For example, Dumas (2011), citing Barnett (1975) highlights that the species can be toxic to turkeys, where a 38 % mortality is recorded in birds who diet consisted of 10 % of *P. americana* seeds (data from the USA). Additionally, Dumas (2011) detail that mortality has been recorded in pigs, cows and horses.

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	**minor**	**CONFIDENCE**	**medium**

Comments: All known and potential impacts are listed in the previous categories.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	**minimal**	**CONFIDENCE**	**medium**

Comments:
There is no information on this issue.

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	**minimal**	**CONFIDENCE**	**medium**

Comments: The previous sections have covered all impacts known for the species and other impacts are likely to be minimal.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comments: The expected impacts of *P. americana* would remain the same within the RA area as there are no natural enemies that would impact on the species. Any generalist natural enemies that do attack the species would not inflict a significant impact on the population.

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	**moderate**	**CONFIDENCE**	**medium**

Comment: The overall impact in RA area in foreseeable climate change conditions is unlikely to change from that of the current climatic conditions. New (northern) areas of the RA area may be suitable for the establishment of the species but it is likely that the impact will remain moderate with a medium confidence to reflect the uncertainty of climate change prediction. Areas in the Mediterranean may become less suitable for the establishment of the species in the natural environment and thus less impact may be seen in these areas.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Introduction*	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	When considering all pathways into the RA area, it is likely that *P. americana* can enter the region with a medium confidence. There are three potential active pathways of introduction: horticulture, release in nature for use and transport -contamination. However, it should be noted that the risk of these pathways is negligible in view of the already established populations of the species in the RA area.
Summarise Entry*	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	When considering all pathways for entry into the RA area, it is likely that *P. americana* can enter the natural environment, with a medium confidence.
Summarise Establishment*	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	The species is already established within the RA area and further establishment in climatically similar environments is likely. Habitats within the RA area are widespread.
Summarise Spread*	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	The species can spread by human assisted and natural spread. Both are major spread pathways for the species within the RA area.
Summarise Impact*	minimal minor **moderate** major massive	**low** medium high	The species can form dense monocultures which can act to outcompete native plant species. However, the species mainly invades ruderal habitats of low conservation importance.
Conclusion of the risk assessment (overall risk)	low **moderate** high	low **medium** high	There are active pathways where the species can enter the RA area, and the natural environment. The species is capable of establishing in the RA area and spreading moderately. The impact of the species needs further research, but is within this RA considered as moderate. Based on these scores the overall assessment of risk is moderate with a medium uncertainty.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	YES	YES	YES	YES	
Belgium	YES		YES	YES	
Bulgaria	YES	YES	YES	YES	
Croatia	YES	YES	YES	YES	
Cyprus	YES	YES	YES	YES	
Czech Republic	YES	YES	YES	YES	
Denmark				YES	
Estonia			YES	YES	
Finland			YES	YES	
France	YES	YES	YES	YES	YES
Germany	YES	YES	YES	YES	
Greece	YES	YES	YES	YES	
Hungary	YES	YES	YES	YES	YES
Ireland					
Italy	YES	YES	YES	YES	YES
Latvia			YES	YES	
Lithuania			YES	YES	
Luxembourg			YES	YES	
Malta			YES	YES	
Netherlands	YES	YES	YES	YES	
Poland	YES	YES	YES	YES	YES
Portugal	YES	YES	YES	YES	YES
Romania	YES	YES	YES	YES	YES
Slovakia	YES	YES	YES	YES	
Slovenia	YES	YES	YES	YES	YES
Spain	YES	YES	YES	YES	YES
Sweden				YES	
United Kingdom	YES			YES	

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine				YES	YES
Atlantic	YES	YES	YES	YES	YES
Black Sea	YES	YES	YES	YES	
Boreal	YES	YES	YES	YES	
Continental	YES	YES	YES	YES	YES
Mediterranean	YES	YES	YES	YES	YES
Pannonian	YES	YES	YES	YES	YES
Steppic	YES	YES	YES	YES	

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[7]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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[7] Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.* 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy

			Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[8]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires*

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[8] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			etc.
		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences

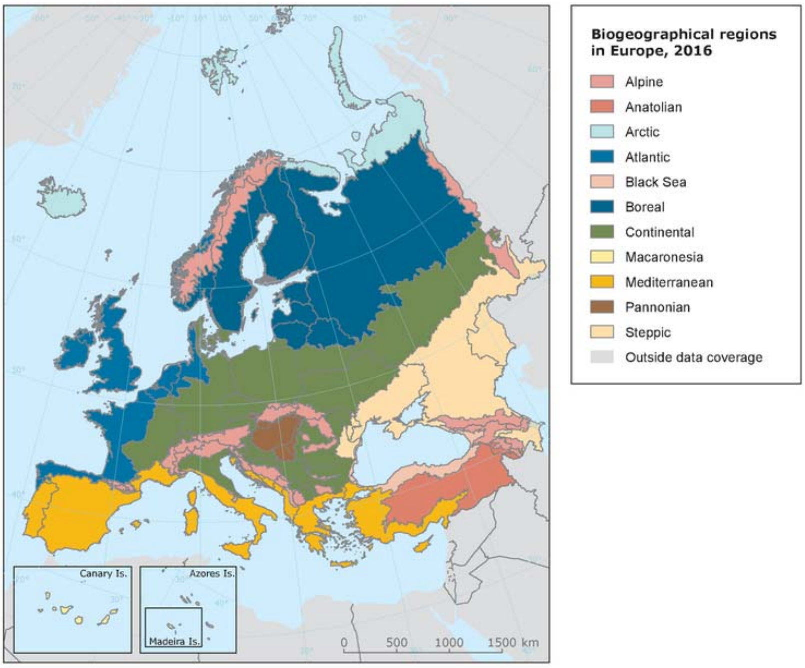
			Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

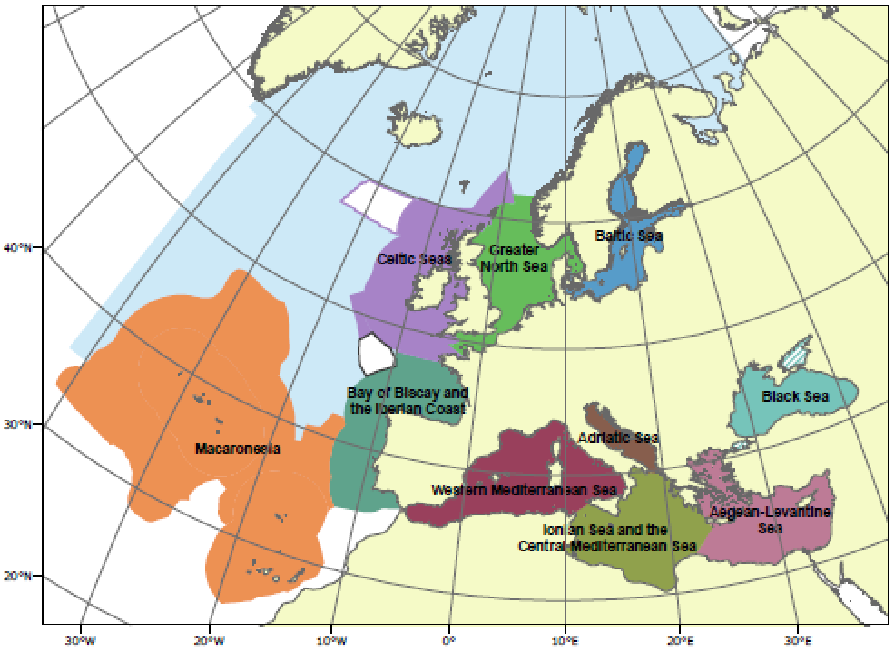
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

ANNEX VII Projection of climatic suitability for *Phytolacca americana* establishment

Björn Beckmann, Rob Tanner, Richard Shaw, Beth Purse and Dan Chapman

30 October 2019

Aim

To project the suitability for potential establishment of *Phytolacca americana* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (18598 records), the Biodiversity Information Serving Our Nation database (BISON) (6638 records), the Integrated Digitized Biocollections (iDigBio) (1327 records), the Atlas of Living Australia (179 records), the Berkeley Ecoinformatics Engine database (113 records), and a small number of additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils, captive records) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 4321 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Tracheophyta records held by GBIF was also compiled on the same grid (Figure 1b).

(a) Species distribution used in modelling

(b) Estimated recording effort (log10-scaled)

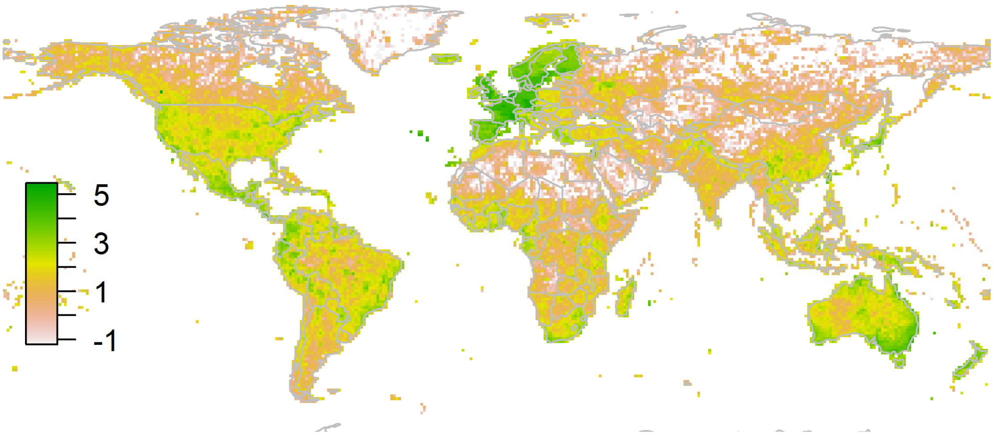


Figure 1. (a) Occurrence records obtained for *Phytolacca americana* and used in the modelling, showing native and invaded distributions. (b) The recording density of Tracheophyta on GBIF, which was used as a proxy for recording effort.

Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Phytolacca americana*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Climatic moisture index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed. For its calculation, monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar radiation using the

simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. There represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1 (Thuiller et al., 2019, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Phytolacca americana* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Phytolacca americana* at the spatial scale of the model:
    - Minimum temperature of the coldest month (Bio6) < -27
    - Mean temperature of the warmest quarter (Bio10) < 3
    - Climatic moisture index (CMI) < log1p(0.20)

Altogether, only 0.2% of occurrence grid cells were located in the unsuitable background region.

Within the background region, 10 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2).

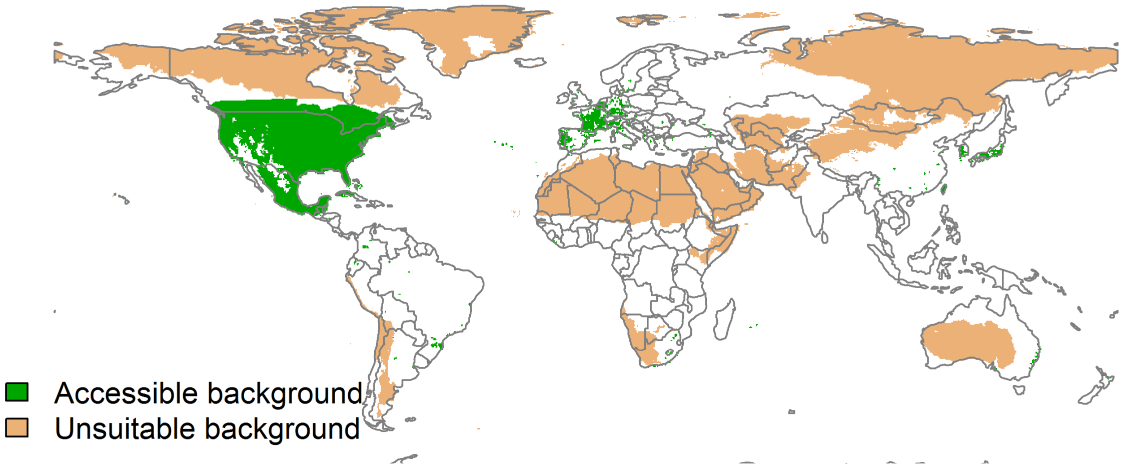


Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Phytolacca americana*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 1(b)).

Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCdist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Phytolacca americana* was most strongly determined by Climatic moisture index (CMI), accounting for 40.1% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (31.2%) and Minimum temperature of the coldest month (Bio6) (28.8%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

					variable importance (%)		
Algorithm	AUC	Kappa	TSS	Used in the ensemble	Climatic moisture index (CMI)	Mean temperature of the warmest quarter (Bio10)	Minimum temperature of the coldest month (Bio6)
GLM	0.902	0.611	0.647	yes	42	32	26
GAM	0.902	0.609	0.644	yes	40	30	30
ANN	0.906	0.607	0.653	yes	38	32	30
GBM	0.906	0.604	0.649	yes	41	30	29
MARS	0.901	0.603	0.642	yes	43	30	27
RF	0.842	0.480	0.580	no	36	36	29
Maxent	0.901	0.593	0.635	yes	35	34	30
Ensemble	**0.906**	**0.608**	**0.650**		**40**	**31**	**29**

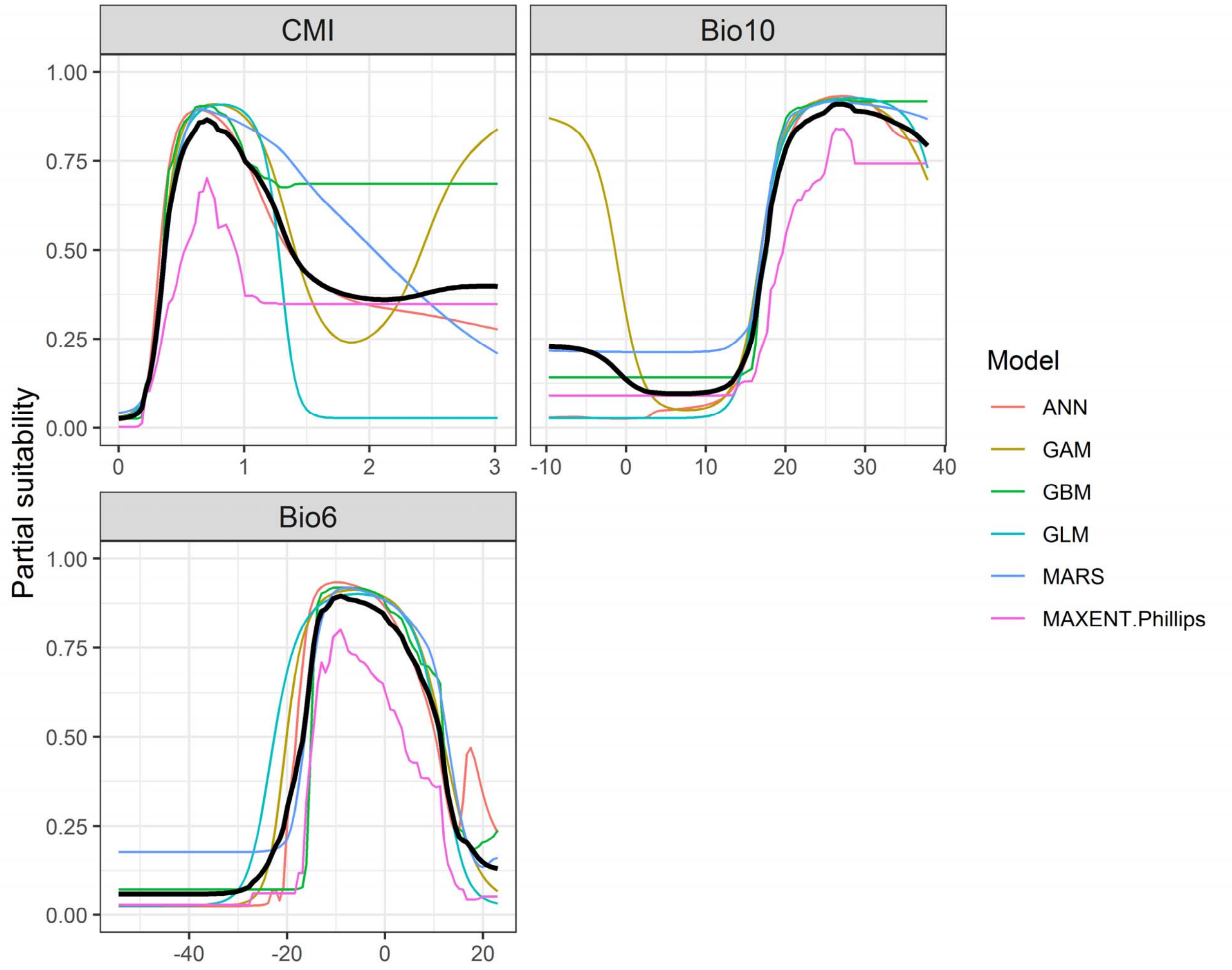
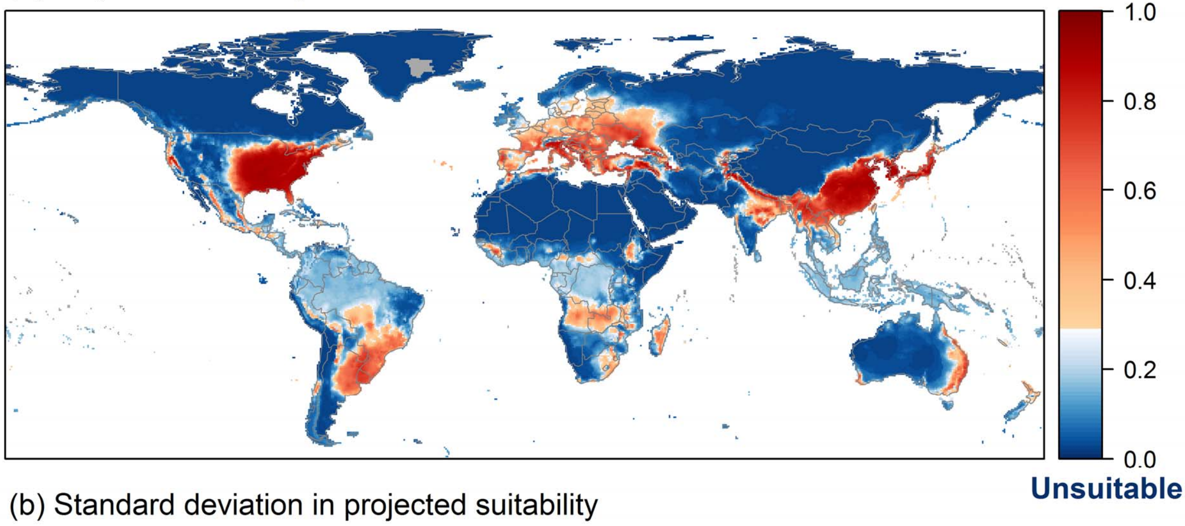


Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

(a) Projected suitability



(b) Standard deviation in projected suitability

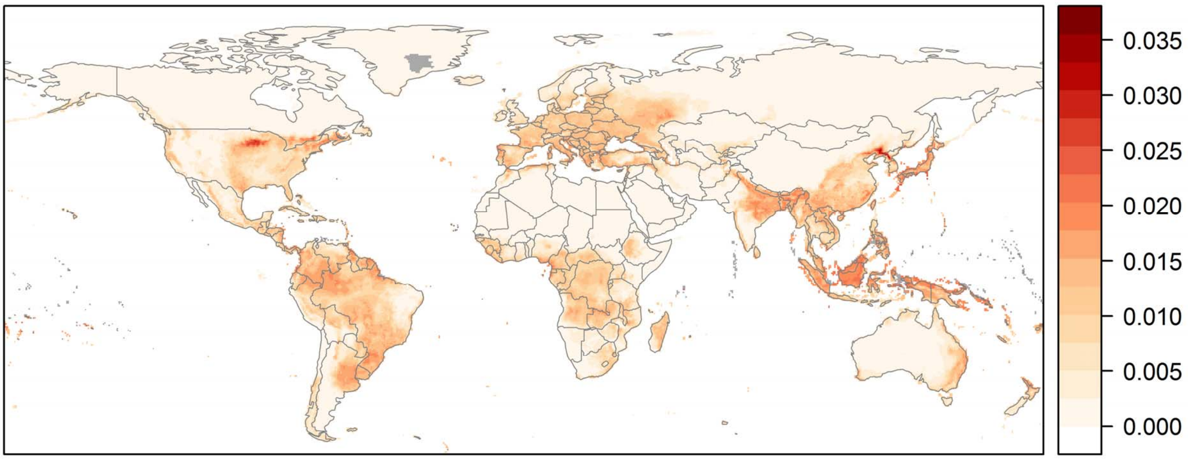
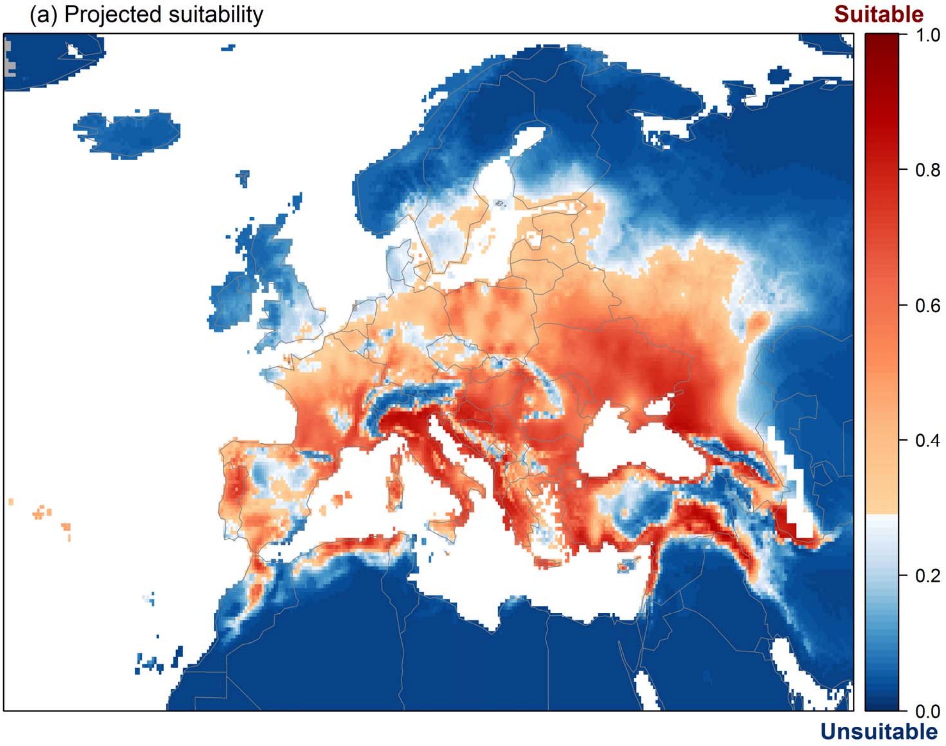


Figure 4. (a) Projected global suitability for *Phytolacca americana* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.28 may be suitable for the species. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

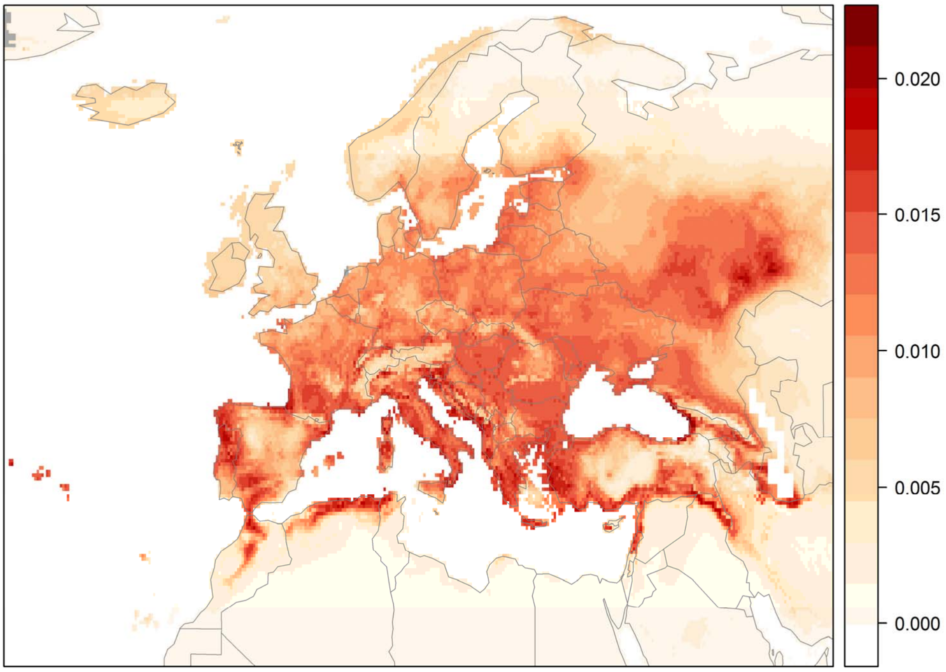


Figure 5. (a) Projected current suitability for *Phytolacca americana* establishment in Europe and the Mediterranean region. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

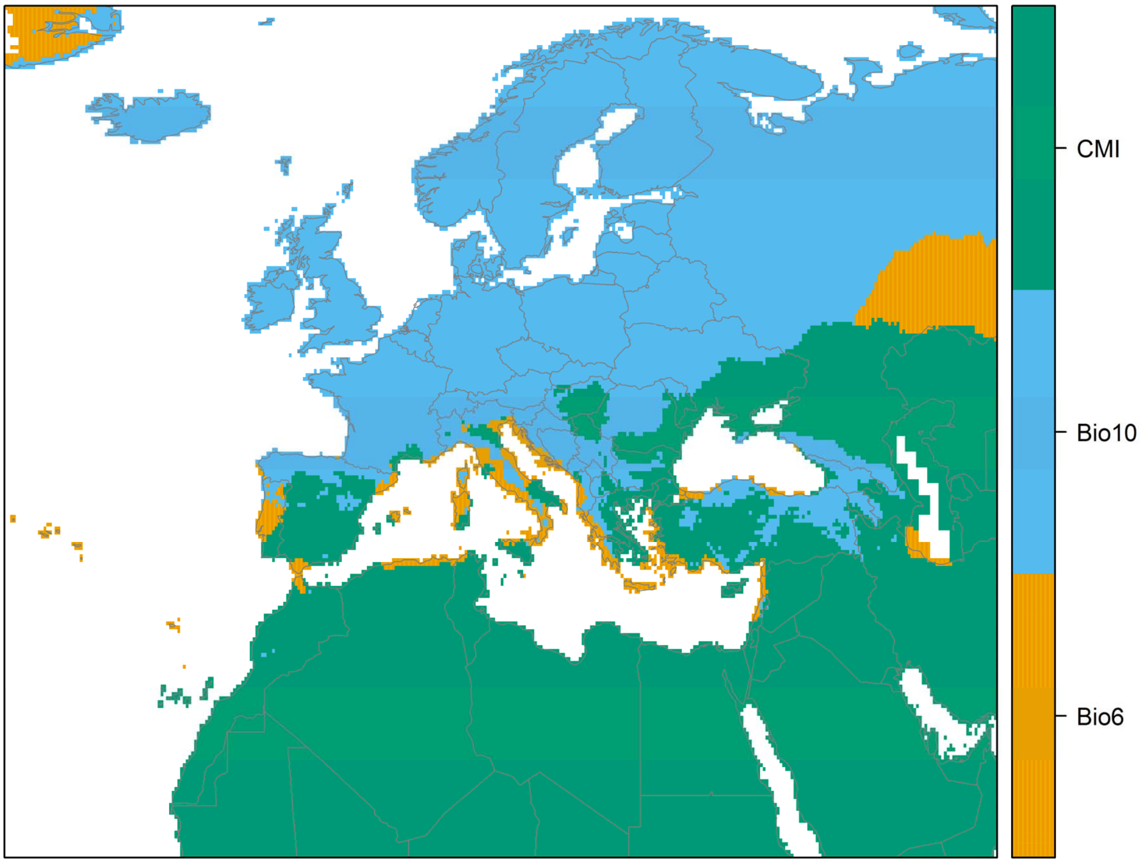
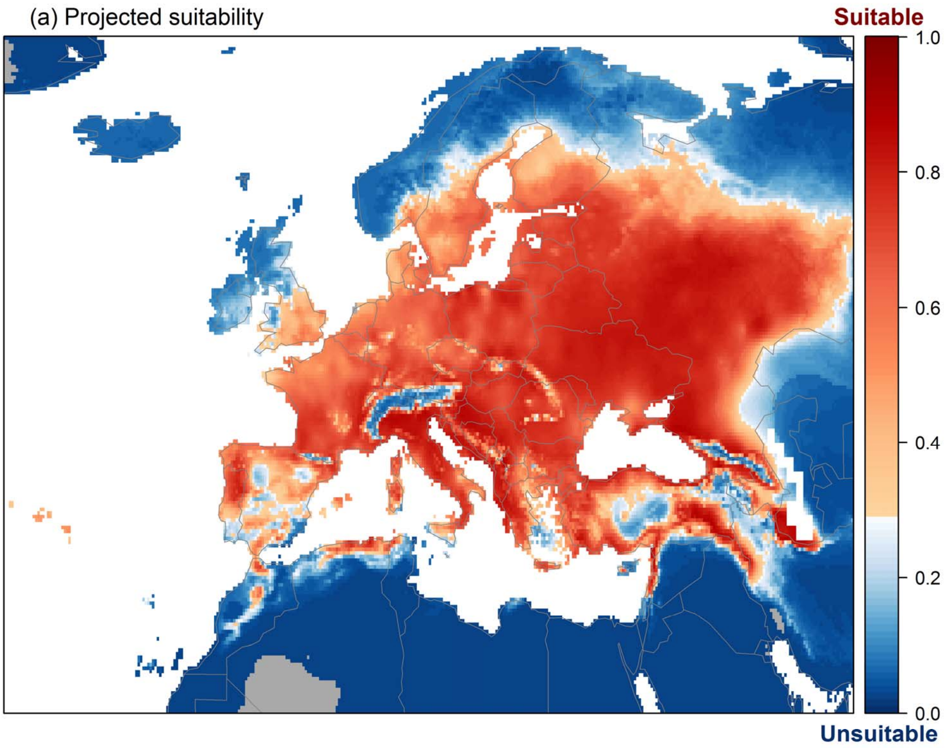


Figure 6. The most strongly limiting factors for *Phytolacca americana* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

(a) Projected suitability



(b) Standard deviation in projected suitability

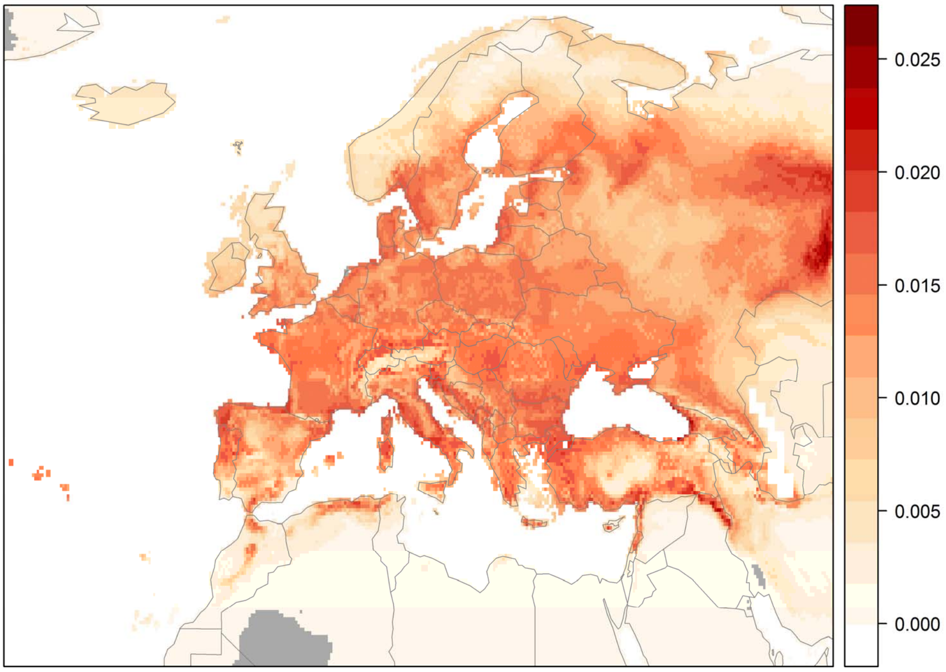
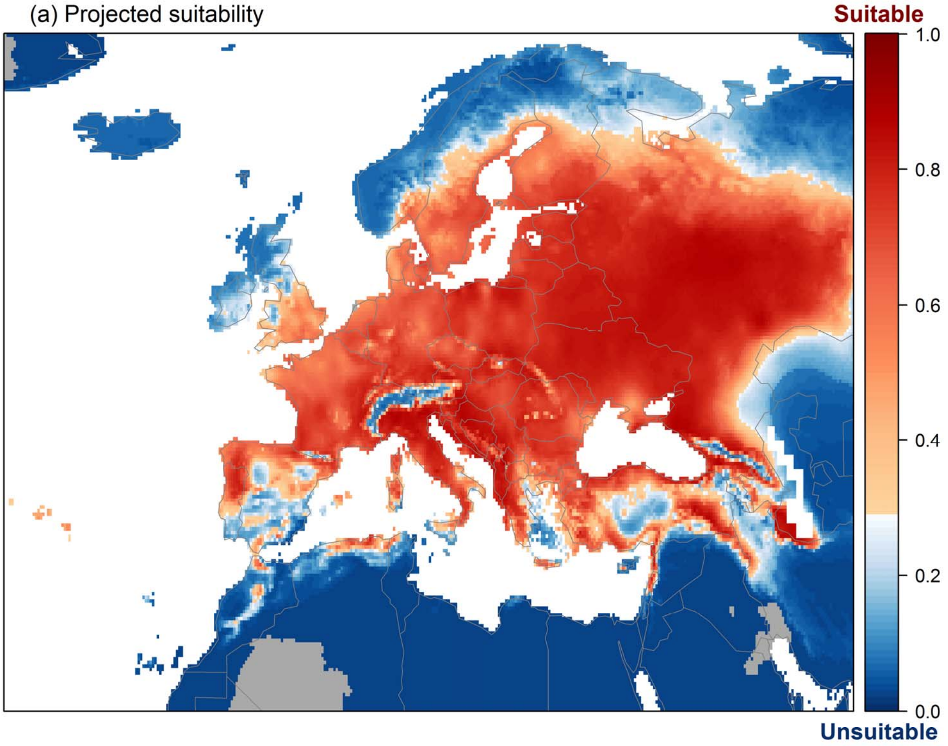


Figure 7. (a) Projected suitability for *Phytolacca americana* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

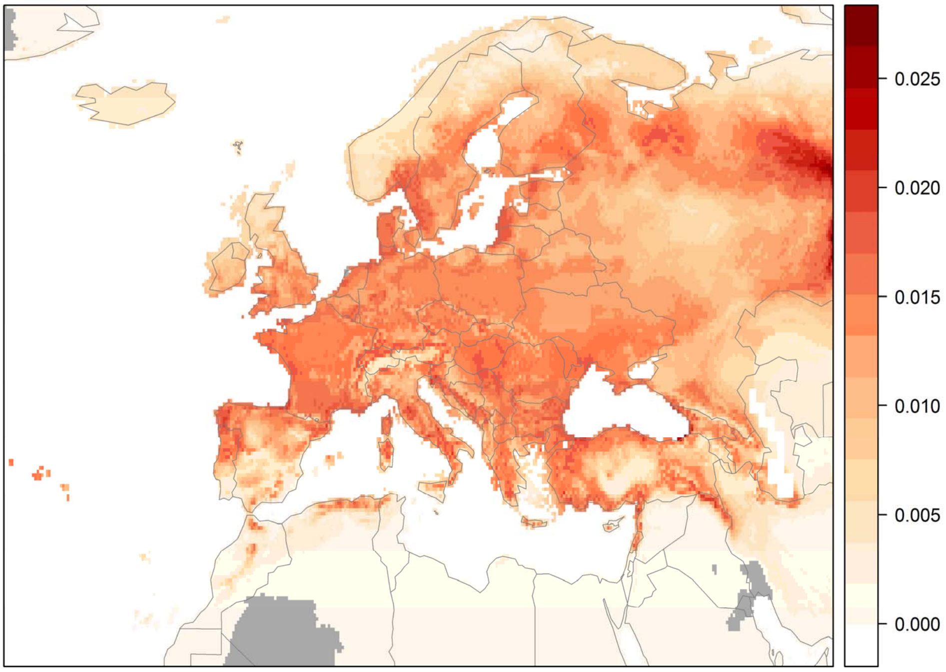


Figure 8. (a) Projected suitability for *Phytolacca americana* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

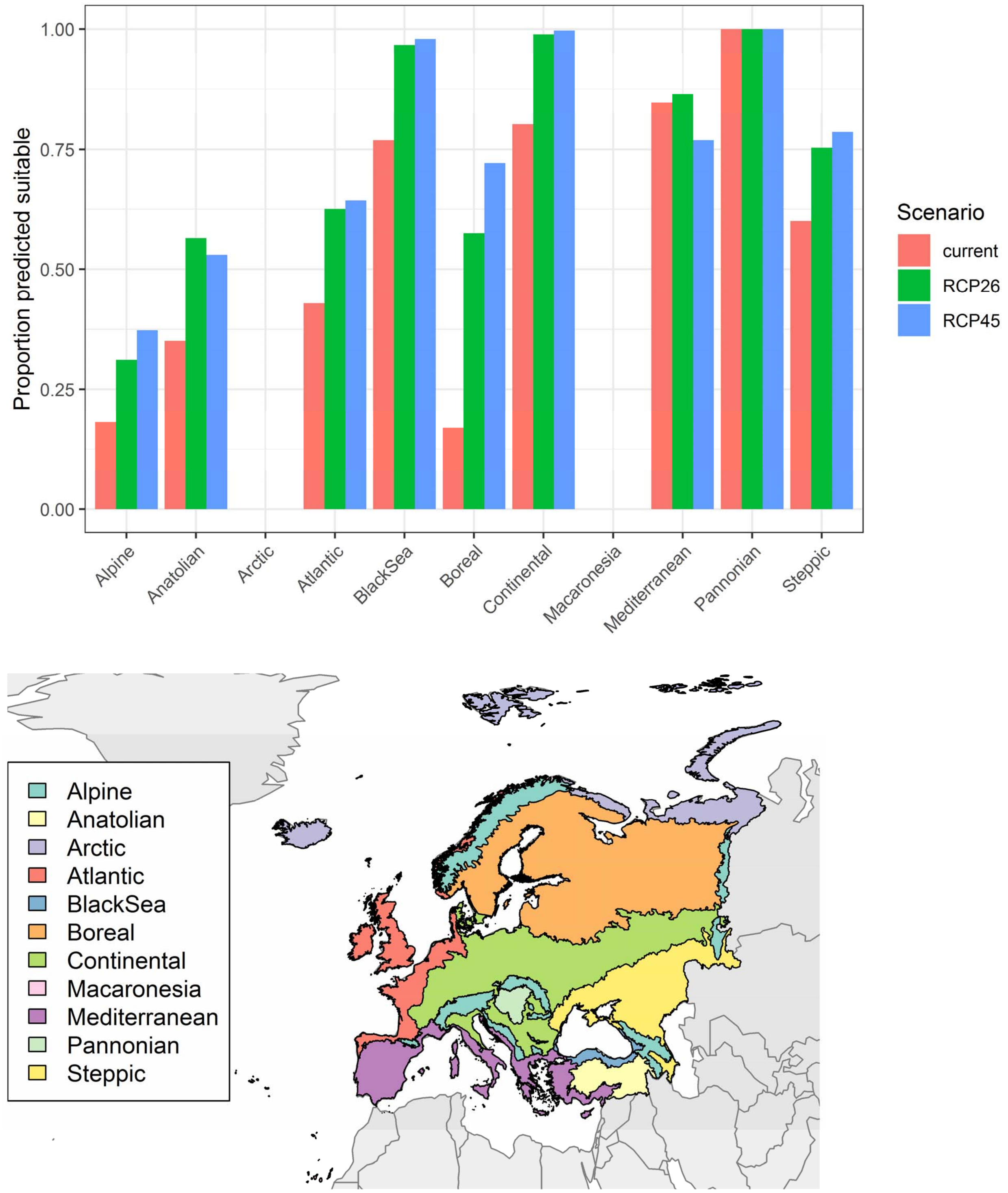


Figure 9. Variation in projected suitability for *Phytolacca americana* establishment among Biogeographical regions of Europe (Bundesamt für Naturschutz (BfN), 2003). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness (although unsuitable for all scenarios for this species).

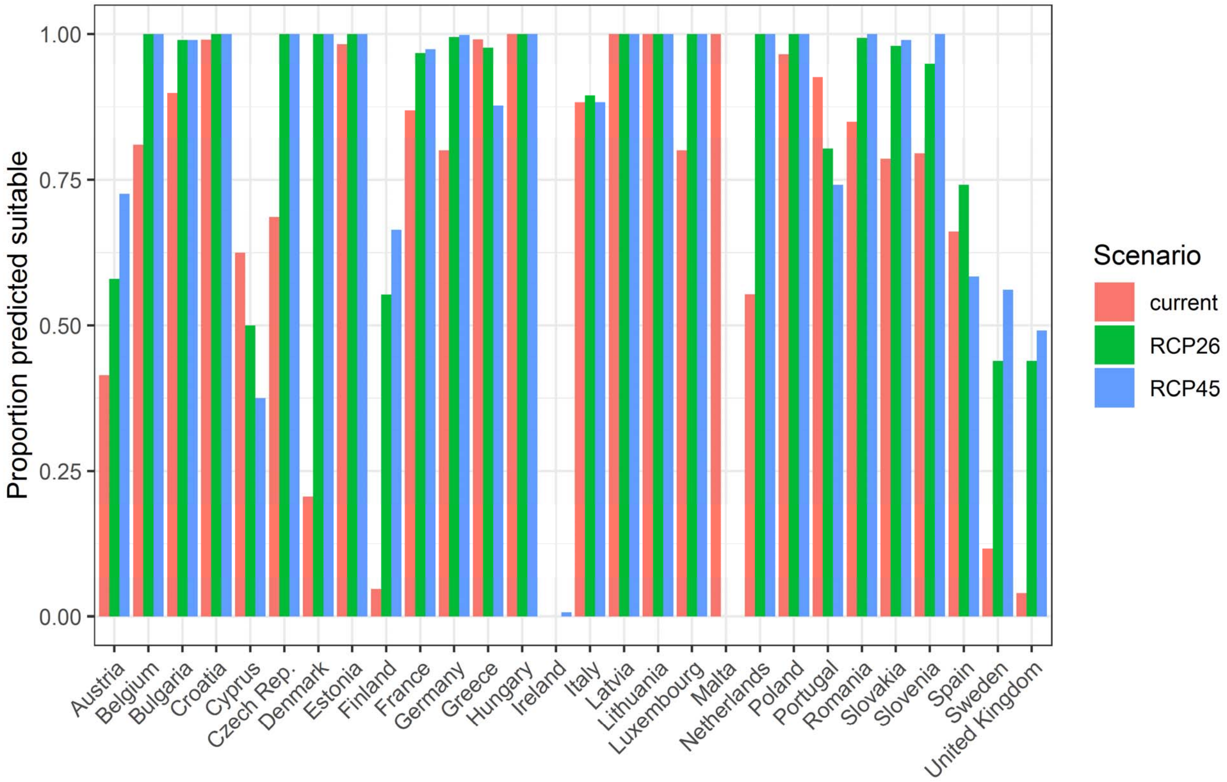


Figure 10. Variation in projected suitability for *Phytolacca americana* establishment among European Union countries. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

To remove spatial recording biases, the selection of the background sample was weighted by the density of Tracheophyta records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Phytolacca americana* L.**
Species (common name)	**American pokeweed**
Author(s)	**Rob Tanner**
Date Completed	**5.07.2019**
Reviewer	**Peter Robertson**

Summary [1]

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

***Phytolacca americana* is already established within the risk assessment area and although the pathways of horticulture (escape from confinement), transport (contamination (transport of habitat material: soil and vegetation)) and stowaway on used machinery and equipment are detailed in the RA, the overall likelihood of the species entering via these pathways is moderate. Thus, to mitigate the impact of this species within the RA area, measures should focus on early detection and eradication of the species where it occurs.**

***Phytolacca americana* is a perennial herb. Spread of the species is predominantly by the spread of seeds by natural means from areas where the species has been planted. There is also the potential of root fragments being spread following disturbance of areas where the species occurs. Although there is no data on the growth rates of the species, the species can grow vigorously and produce many seeds which can become incorporated into a seedbank which can persist for a number of years.**

Although there are no specific studies on the management of *P. americana* in the EU it can be considered that management practices should follow that of other perennial species that form persistent seed banks. *P. americana* can be managed with traditional methods including the use of physical and mechanical methods, and the utilisation of chemical control methods. Physical and mechanical methods can include the utilization of various machinery and tools (mowers, shears etc) and the physical pulling of young plants. Repeated applications are likely to be required to exhaust the long-lived seedbank. Chemical control methods can be effective against the species though repeated measures would be needed as the species is perennial and one application may not be enough.

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)[3]**	**Level of confidence[4]**
Methods to achieve prevention [5]	One potential pathway for entry or spread of *Phytolacca americana* into the EU, or between Member States, is through the purchase of material through the horticultural trade. A ban from sale would help to regulate this pathway for the species. The species is also traded between Member States via internet suppliers.	*Phytolacca americana* is not a significantly popular species in trade and therefore a ban on the sale of the species would be a reasonably cost-effective measure at preventing the movement, both from outside and within the RA area. A ban from sale requires resources including financial resources, staff time and the development of communication material from a number of sectors, including governmental, regulators, horticulture and horticultural suppliers, the general public, and environmental NGOs. Communication material detailing the negative impacts of the species would be essential to educate the public and support a ban on sale. Public awareness campaigns may highlight the risk of the species and prevent further spread of the species from existing populations. *Phytolacca americana* does have some uses (it has been used as a food plant and for other medicinal purposes) and therefore communication material should also address this aspect highlighting the negative impacts compared to the few benefits the species has. It is estimated that the cost for an awareness raising campaign could be up to EUR 10,000 per year (which would include the cost to produce and disseminate information material along with associated staff costs) for each Member State.	Moderate confidence in the assessment

	The transport of seed material as a contaminant of habitat material (soil and vegetation) may facilitate its entry into and spread within the EU. Phytosanitary inspections along with associated phytosanitary measures can act to prevent the entry of the species into specific countries/regions. To prevent the movement of contaminated material between EU Member States, management plans for habitat material, identification guides, factsheets, Codes of conduct should be referred too/developed. Preventing the spread of *P. americana* should also be regarded as a priority to limit further invasive populations. Measures to achieve this are listed in the eradication and management sections. Preventing the establishment of P. *americana* should be the priority as eradication can be difficult and complicated and may only be feasible over limited areas and during early phase of establishment.	Phytosanitary inspections can be implemented on commodities coming into the EU from outside but the risk of *P. americana* entering as a contaminant is moderate as the seed is small and can be hidden within material. The author could not find any examples where seed has been intercepted as a containment. It is however, very difficult to implement phytosanitary measures within the EU due to freedom of movement of commodities between countries. If measures are not implemented by all countries, they will not be effective since the species could spread from one country to another. National measures should be combined with international measures, and international coordination of management of the species between countries is recommended.	Moderate confidence in the assessment
	The transport of seed material as a stowaway on used machinery and equipment may facilitate its	Phytosanitary inspections can be implemented on machinery and equipment coming into the EU from outside but the risk of *P. americana* entering as a contaminant is moderate as the seed is small and can be	Moderate confidence in the assessment

	entry into and spread within the EU. Phytosanitary inspections along with associated phytosanitary measures can act to prevent the entry of the species into specific countries/regions. To prevent the movement of machinery and used equipment between EU Member States, biosecurity measures can be applied, and reference should be made to international Standards such as ISPM 41 (International movement of used vehicles, machinery and equipment). Management plans for habitat material, identification guides, factsheets, Codes of conduct should be referred too/developed. Preventing the spread of *P. americana* should also be regarded as a priority to limit further invasive populations. Measures to achieve this are listed in the eradication and management sections. Preventing the establishment of P. *americana* should be the priority as eradication can be difficult and complicated and may only be feasible over limited areas and during early phase of establishment.	hidden within habitat material. The author could not find any examples where seed has been intercepted as a containment. Cleaning of machinery and equipment should be implemented before it is moved to new areas to avoid the movement of seed material from infested sites. If measures are not implemented by all countries, they will not be effective since the species could spread from one country to another. National measures should be combined with international measures, and international coordination of management of the species between countries is recommended.	

Methods to achieve eradication [6] There are a number of methods that can potentially achieve eradication of discrete populations. However, if the population occurs along riverbanks or over a large area eradication attempts may be limited or will require investment over a number of years. It is also important to note that any eradication method may need to be used in combination, for example removal of the above-ground foliage will not achieve eradiation alone and will need to be used in combination with excavation of the below-ground rhizomes or repeated chemical application to deplete the below-ground biomass. Eradication methods can be applied on a local scale. Methods would depend on the habitat where the species is invasive and the extent of the infestation.	**Manual control using mechanical or manual removal**	Mechanical and manual control can take the form of cutting using basic hand-held non-motorised utensils or motorised machinery such as mowers or strimmers (Fu *et al*., 2012) Larger agriculture machinery may be used in more open habitats. Hand pulling can be used to remove the aboveground material though protective clothing should be worn. If the substrate is compact, handpulling should take place during time of rain to facilitate the removal of the root system. Hand pulling may only be effective on small plants as once plants have established, they can develop an extensive root system. Such measures may be effective at removing the above ground biomass of the species, but would be relatively ineffective at exhausting the rhizomes, and seed bank which can remain viable for up to 40 years (Vuillemenot & Mischler, 2012). Rhizomes can be difficult to remove from the soil as they can break easily leaving viable fragments in the soil. Using a spade can help this issue and may ensure all belowground material is removed. Effective removal of the above and belowground plant material is essential for the eradication of the species and this can be labour intensive. No monetary figures have been found on the manual control for *Phytolacca americana*. However, if considering costs may be similar for P. americana to that of manual control of giant hogweed (Rajmis et al., 2017) costs could range from 20 – 40 euros per hour, plus additional costs for training and protective equipment. Depending on the size of the infestation and the habitat costs may be substantial to eradicate the species. Removing a small number of plants from a limited area (<1ha) may be feasible (1000 -2000 Euros). However, at larger scales and in more complex environments, feasibility of eradication is likely to drop rapidly	Moderate confidence in the assessment

		with significant increases in cost. However, little evidence is available on which to base further assessments.	
	Chemical application (herbicides)	*Phytolacca americana* is a perennial species and therefore a single chemical application would not provide effective control. Foliar application of herbicides (e.g. active ingredient glyphosate) can be used. The timing of application should occur when the plants are post emergence and growing rapidly. Application in mid-to late summer can provide the best control (DiTomaso et al., 2013). Dumas (2011) also highlight triclopyr as a potential herbicide. Stem injections may also be a suitable option to target individual plants though this can increase the costs for labour per Ha. The weeding of this species is difficult in maize crop where herbicide spraying is only efficient on seedlings, but not on regrowth, and it requires an additional spray increasing the weed management cost (Mamarot & Rodriguez, 2003). Villemenot & Mischler (2012) also indicate that in forest, *P. americana* requires more regular clearance work until the top of the young trees exceeds *P. americana*.	High confidence in the assessment
	Removal of root material and soil contaminated with roots or soil	Excavating soil contaminated with root material or seed material could potentially assist in eradication attempts but may be costly as heavy machinery may be needed and the contaminated soil should be removed to a licenced landfill which will incur additional costs. Removing seed *in situ* is unlikely to be a feasible method.	Moderate confidence in the assessment
	Covering with black plastic	Although there is no research conducted on eradicating the species by using black plastic, this method may be useful for small areas. Covering the soil where the above ground plant material has been cleared could act to prevent the emerging plant material from attaining sunlight and thus could act to deplete the resources below ground. However, it is unclear if this will act to exhaust the seed bank which can germinate upon disturbance.	Moderate confidence in the assessment

Methods to achieve management [7]		See methods 1-4 in 'methods to achieve eradication' which can all be used to achieve management.	
Management methods can be applied on a local scale. Methods would depend on the habitat where the species is invasive and the extent of the infestation.	**Manual control**	Manual control alone may not be considered to be a cost-effective option for long-term management as repeated measures would be needed and control would need to take place over a number of years in order to exhaust the seed bank (Dumas, 2011).	High confidence in the assessment
	Chemical control	Chemical control could be considered cost effective when controlling small populations of the species in discrete areas. Repeated applications may be needed to maintain suppression of the population.	High confidence in the assessment
	Excavation of the roots and seed from the soil	Excavation of roots and seeds from the soil, and contaminated soil may be cost effective over a long period of time but the initial outlay of costs can be high and include costs of heavy machinery and costs of disposal of contaminated soil. Further complications can arise depending on the habitat type invaded, where for example invasions into natural habitats and managed forests may act to increase the costs associated with management. Removing a small number of plants from a limited area (<1ha) may be feasible and relatively inexpensive (1000-2000 Euros). However, at larger scales and in more complex environments, feasibility of eradication is likely to drop rapidly with significant increases in cost. However, little evidence is available on which to base further assessments.	High confidence in the assessment
	Covering with black plastic	Although there is no research conducted on managing the species by using black plastic, this method may be useful for small areas. Covering the soil where the above ground plant material has been cleared could act to prevent the emerging plant material from attaining sunlight and thus could act to deplete the resources below ground. However, it is unclear if this will act to exhaust the seed bank which can germinate upon disturbance.	Moderate confidence in the assessment

	Biological control	At present, biological control against *Phytolacca americana* has not been considered (Dumas 2011). However, the cost-effectiveness of instigating and delivering a classical biological control programme against this species would initially be low as considerable costs would be needed to fund the control programme. A classical biological control programme can cost in the region of 600,000 EUR.	High confidence in the assessment

References

DiTomaso JM, Kyset GB et al., (2013) Weed control in natural areas in the western United States. Weed Research and information Center, University of California, 544 pp.

Dumas Y. (2011) Que savons-nous du Raisin d'Amérique (Phytolacca americana), espèce exotique envahissante ? Synthèse bibliographique. Rendez-vous techniques ONF, 2011, p. 48 - p. 57. hal- 00672349

Fu, J.P., Li, C.R., Xu, J.W., Cheng, W.L., Song, R.F. and Liu, Y., 2012. Prevention and control of invaded plant Phytolacca americana in sandy coastal shelter forests. *Ying yong sheng tai xue bao= The journal of applied ecology*, *23*(4), pp.991-997.

Rajmis, S., Thiele, J. and Marggraf, R. (2017). A cost-benefit analysis of controlling giant hogweed (Heracleum mantegazzianum) in Germany using a choice experiment approach. NeoBiota 31:19–41

Vuillemenot M. & Mischler L., 2012. *Le raisin d'Amérique (*Phytolacca americana *L.) en Franche-Comté ; bilan stationnel et proposition d'un plan régional de lutte.* Conservatoire botanique national de Franche-Comté – Observatoire régional des Invertébrés, 69 p. + annexes.

Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; this is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism:

Boccardia proboscidea Hartman, 1940

Author(s) of the assessment:

- Marika Galanidi, Dr, Dokuz Eylul University, Institute of Marine Sciences and Technology, Izmir, Turkey
- Argyro Zenetos, Dr, Research Director, Hellenic Centre for Marine Research, Greece
- Björn Beckmann, Dr, Centre for Ecology & Hydrology, Edinburgh, UK

Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: Vasily Radashevsky, National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, Russia

Peer review 2: Jack Sewell, The Marine Biological Association, UK

Date of completion: 01/11/2019

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:

Phylum: Annelida, **Class**: Polychaeta, **Order**: Spionida, **Family**: Spionidae

Boccardia proboscidea **Hartman, 1940**

A burrowing spionid worm

Synonym: *Polydora californica* (Treadwell, 1914)

Spio californica (Fewkes, 1889)

Polydora californica (Treadwell, 1914) and *Spio californica* (Fewkes, 1889) were both suppressed in 2012 by the International Commission on Zoological Nomenclature (ICZN, case 3520). The widely cited and used name, *Boccardia proboscidea* (Hartman, 1940) was conserved (ICZN, 2012).

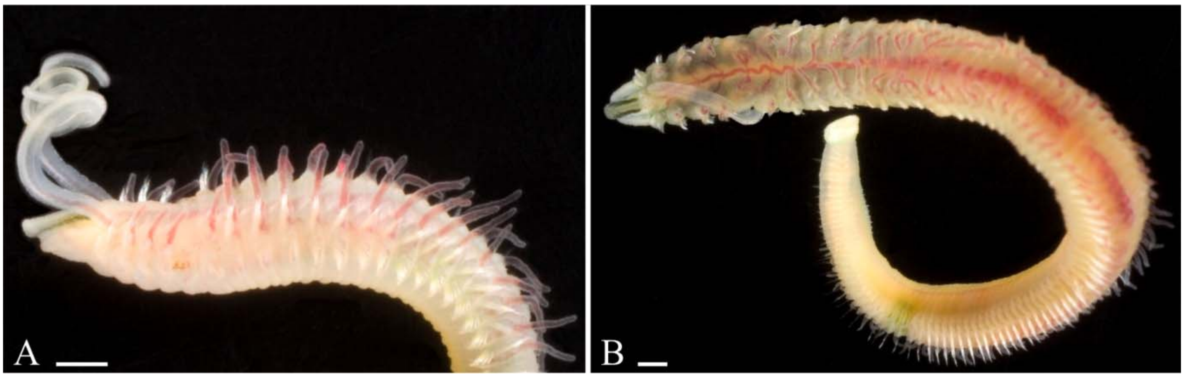


Image: Adult *B. proboscidea*. A, anterior end, left lateral view. B, complete individual. Scale bars: A, B – 500 µm. Photos by Leon Altoff, published in Radashevsky *et al*. (2019) and included with permission by the publisher.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: Spionid polychaete worms are common shellfish pests throughout the world, especially species of Polydora and Boccardia which are commonly referred to as "mudworm" (Handley, 1995). Among the most widespread spionid species in Europe worth mentioning are *Polydora ciliata (*native in NW Europe*)* and *Polydora cornuta* (cosmopolitan, alien). For differences among the spionid species see Rainer (1973). There are three *Boccardia* species in European waters (*Boccardia polybranchia* (Haswell, 1885)*, Boccardia proboscidea* and *Boccardia semibranchiata* Guérin, 1990) which can be misidentified with the two *Boccardiella* species (*Boccardiella hamata* (Webster, 1879) and *Boccardiella ligerica* (Ferroniere, 1898)). The genera Boccardia and Boccardiella differ in the presence of branchiae on the first setigers: in Boccardiella they are present on setigers 2, 3 and 4, in Boccardia branchiae are present only on setigers 2 and 3 (Blake & Kudenov, 1978). Moreover, these two genera differ in the kinds of modified setae on the fifth setiger, *Boccardiella* species have one (simple and falcate) while *Boccardia* have two (one simple and falcate and the second expanded and club-like) (Blake & Ruff 2007).

Identification of this species with a recent regional polychaete identification manual (Hartmann-Schroder, 1996) readily points to *Boccardiella ligerica* due to the gills on chaetigers 2, 3 and 4. Identification with the older and more southerly-oriented manual of Fauvel (1927) points to *Boccardia polybranchia* (Kerckhof & Faasse, 2014). Martınez *et al*. (2006) provide a key to *Boccardia* species recorded from the Atlantic, including *B. proboscidea,* which highlights the short notopodial chaetae on the first chaetiger of *B. proboscidea* that are lacking in *B. polybranchia*. *Boccardia proboscidea* further differs from other Boccardia species recorded from the Atlantic, in the bristle-tipped special chaetae of chaetiger 5 without subdistal bosses and the undivided prostomium. *Boccardia proboscidea* has *a* robust, thick body reaching 45 mm length (Radashevsky *et al*., 2019).

Boccardia polybranchia is green to reddish-yellow in color and has a notched prostomium. Its first setiger lacks notosetae, it has only 60–80 segments and a pygidium that is a thick ring. *Boccardia polybranchia* is a cosmopolitan species that lives in estuarine mud and is reported in France (Ruellet , 2004) and UK , yet with reservations about its correct identification.

Boccardia semibranchiata is a recently described species from the Mediterranean Sea. It is reported from the Atlantic France (Goulletquer *et al*., 2002) and Spain (Martínez *et al*., 2006).
Boccardiella hamata has recurved spines, rather than straight bifid uncini, on its posterior parapodia and the pygidium has two lappets (Hartman 1969). It is common in oyster beds and builds tubes in mudflats or bores holes into hermit crab and bivalve shells and is reported from the Netherlands (Kerckhof & Faasse, 2014). *Boccardiella hamata* can be easily confused and has been misidentified a*s Boccardiella ligerica*), a cryptogenic species reported from the Baltic Sea and North Sea (Jansson,1994).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: No full risk assesment is available. However David *et al* (2016) have studied its dispersal potential is South Africa. Results obtained from David *et al* (2016) genetic study and dispersal simulations have shown that *B. proboscidea* can disperse and become established along a large section of the South Africa coast irrespective of biogeographic boundaries. Its success will be due in part to its versatile reproductive strategy and wide thermal tolerance. However, it appears that anthropogenic movement will most likely be the critical factor governing both the spread and long-term establishment of this species in southern Africa.

Garaffo *et al* (2016) have povided predictive models for variations in the density of *B. proboscidea* around sewage discharges of Mar del Plata, Argrentina, using environmental variables.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:

Boccardia proboscidea is a eurythermal and euryhaline species (Hartmann, 1940) with a cosmopolitan distribution in temperate seas
Boccardia proboscidea Hartman, 1940 was originally described from northern California (see Radashevsky & Harris, 2010; Fauchald *et al.*, 2011). It is widely reported from the E. Pacific (coast of North America from British Columbia, Canada, south to southern California), and also from the NW Pacific (Japan, Korea and China).

The native distribution of *B. proboscidea* had been uncertain. Based on the type locality only, the species was assumed to be native to California and introduced from British Columbia to Baja California and all other locations (Jaubet *et al.*, 2018), or native to the Pacific coast of North America and Japan (Spilmont *et al.*, 2018)

The high *16S* haplotype diversity of *B. proboscidea* from the Pacific USA suggests a native distribution for the species in the northern Pacific and subsequent introductions through human activities to other parts of the world (Radashevsky *et al.*, 2019).

Its presence in the Asian North-Pacific is of questionable origin (Sato-Okoshi *et al*., 2000; Abe *et al*., 2019) and assumptions that it is native in Japan are not substantiated by concrete evidence (Radashevsky *et al.*, 2019).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Review of previous reports and our new records of *B. proboscidea* show a wide spread of the species in temperate waters across the globe. A phylogenetic analysis revealed the occurrence of single haplotypes in Europe (haplotype K) and in South Africa, Australia and South America (haplotype A), which suggests a recent foundation event for each. The exclusive occurrence of one or the other of these haplotypes suggests at least two distinct routes of colonization from the original distribution zone (Norh Pacific): one for European locations, and one for the Southern Hemisphere ((Radashevsky *et al*., 2019).

The genetic diversity of *B. proboscidea* in Japan, Korea and China, has not been studied. Its common occurrence along the Chinese coast (Yang & Sun, 1988; Zhou *et al.*, 2010) may be indicative of a wider distribution (East and west North Pacific). However the absence of a continous distribution along the North East Pacific may be due to a recent introduction.

Outside of the North Pacific, *B. proboscidea* was first reported from Australia by Blake & Kudenov (1978). Based on this, *B. proboscidea* was suggested to be introduced to Australia with ballast waters discharged in Port Phillip Bay, which hosts the largest seaport in Australia (Pollard & Hutchings, 1990). In 1979, *B. proboscidea* was collected in Elliston, South Australia 1,000 km west of Port Phillip Bay (Hutchings & Turvey, 1984). Therefore, either there were multiple introductions of *B. proboscidea* to southern Australia, or *B. proboscidea* was introduced much earlier and dispersed locally but remained undetected until the first major environmental surveys occurred in the 1970s (Radashevsky *et al*., 2019). The species is widely established, in Argentina (Jaubet *et al*., 2011, 2013, 2015). Jaubet *et al*. (2018) suggested that the species might have been introduced into Argentina from Australia with shipments of Australian bauxite to the aluminum plant in Puerto Madryn that was installed in 1974.

In South Africa, *B. proboscidea* occurs in south-west coasts, indicating establishment in the wild (Simon *et al.*, 2009, 2010; Boonzaaier *et al.*, 2014; David *et al.*, 2016).

Boccardia proboscidea was also introduced to an oyster farm at Keahole, Hawaii, with a shipment of *Ostrea edulis* Linnaeus, 1758 (Bailey-Brock, 2000) from Maine, although no population of the species has been reported there. However, individuals of *B. proboscidea* have not been recorded to date in the wild in Hawaii, even though the species is within its physiological limits there (see Annexes VII & VIII).

Record of *B. proboscidea* from Panama by Fauchald (1977), Mexico (de León-González *et al*., 1993), Chile, by Carrasco (1974, 1976) and Costa Rica (Sibaja-Cordero & Echeverría-Sáenz, 2015) have been refuted by Radashevsky *et al*. (2019) as misidentifications. In most cases (Panama, Mexico, Costa Rica) the records were outside the known thermal tolerance of the species.

In detail sources of distribution with confirmed id.

Australia: Blake & Kudenov, 1978; Hutchings & Turvey, 1984; Pollard & Hutchings, 1990; Thresher & Martin, 1995; Petch, 1995; Hewitt *et al.*, 2004
Argentina (Diez *et al*., 2011; Jaubet *et al*., 2011, 2013, 2015, 2018; Radashevsky, 2011)
South Africa: Simon *et al*., 2009; 2010; David & Simon, 2014; Simon & Booth, 2007; Mead *et al.*, 2011
New Zealand: Read, 2004
Tasmania: Lleonart, 2002
Brazil: Jaubet *et al*., 2018
?Japan: Imajima & Hartman, 1964; Sato-Okoshi, 2000
?Korea: Paik, 1975, 1989
?China: Yang & Sun, 1988; Sun, 1994; Zhou *et al.*, 2010

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

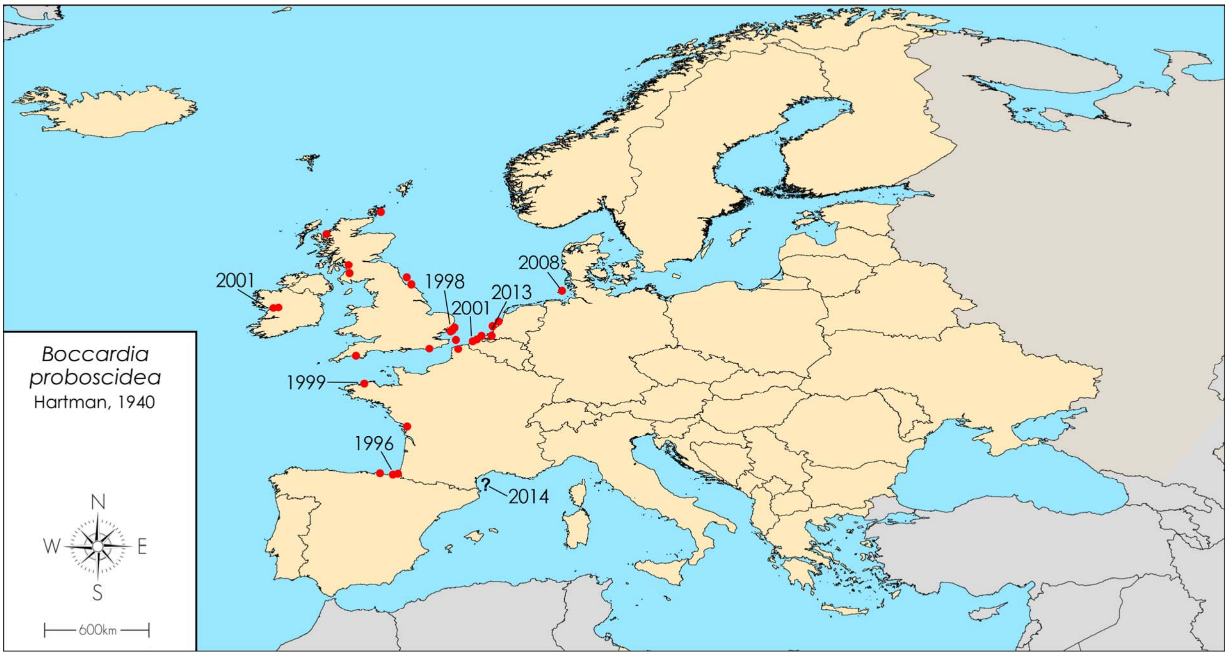
Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf (see also Annex V).



Response (6a): Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea,
Bay of Biscay and the Iberian Coast- established see response 6b
Greater North Sea, incl. the Kattegat and the English Channel: established see response 6b
Celtic Sea: established see response 6b
Western Mediterranean Sea: the discovery of the species in an oyster, purportedly originating in Leucate Lagoon, France (see question mark in the map above) raises concerns for this major aquaculture facility in Mediterranean France. However, there was high uncertainty associated with the origins of the oyster specimen, such that this finding requires confirmation and further investigation (Radashevsky *et al*., 2019).

Response (6b):

Bay of Biscay and the Iberian Coast: established

B. proboscidea was first discovered in 1996 on the rocky intertidal near a sewage outfall at San Sebastián, Bay of Biscay, Spain (Martínez *et al*., 2006) Additional records of the species based on specimens collected in northern Spain during environmental monitoring programs and Studies for Public administrations after 1997. In 2014, *B. proboscidea* was collected from several rocky shores surrounding La Rochelle on the French coast of the Bay of Biscay (Spilmont *et al*., 2018).

Greater North Sea, incl. the Kattegat and the English Channel

In the UK, *B. proboscidea* was first collected in August 1998 on Horsey Island, Essex, south-eastern England. In 2000, worms were found at Shotley, Suffolk, near the ports of Felixstowe, Suffolk, and Harwich, Essex (recorded at generic level in an unpublished report. In 2004 and 2005, it was found in the Tees Estuary, north-eastern England; in 2006, it was found in the Clyde Sea, Scotland and in 2008 in King Edwards Bay, Tynemouth, and Cullercoats Bay, north-eastern England. In 2011, the species was found on rocky intertidal shores in Looe, Cornwall, near Plymouth, and in 2016 it was present in Kent and Sussex, all of which are along the English Channel (full details in Radashevsky *et al*., 2019). In 1999, *B. proboscidea* was collected near Roscoff, Brittany, France (museum specimen #USNM 186423, Radashevsky *et al*., 2019) and in 2014 it was found on an intertidal rocky reef near Wimereux, Hauts-de-France (Spilmont *et al.*, 2018). In the Southern Bight of the North Sea (Belgium), the species was first recorded in 2013 but its presence is suspected since 2001 (Kerckhof & Faasse, 2014 – see also Qu. A8). In the German Bight (Helgoland Island), the first report of *B. proboscidea* (2016) appears in Lackschewitz *et al.* 2017 (in WGITMO, 2017), however the presence of the species backdates to 2008, observed by Ralph Kuhlenkamp and confirmed by Vasily Radashevsky (Radashevsky *et al*., 2019).

Celtic Seas: established

In 2011 and 2013, worms were found intertidally on the Isle of Skye, north-west Scotland (Hatton & Pierce, 2013), and in 2016 we collected them in Great Cumbrae Island, western Scotland (MIMB 33681). Full details in Radashevsky *et al.* (2019)

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): **Combining physiological tolerances and distribution modeling (see Qu 3.1, Qu 3.13 and Annex VII & VIII for details)**

Baltic Sea: unlikely, high confidence
Greater North Sea: very likely, high confidence
Celtic Seas: very likely, high confidence
Bay of Biscay and the Iberian coast: very likely , high confidence
Mediterranean Sea: likely, medium confidence
Black Sea: unlikely, low confidence

Response (7b): **Combining physiological tolerances and distribution modeling** – Estimates in Annex VII refer to a maximum increase in seawater temperatures of 0.8 °C by 2065, according to the medium time frame RCP 4.5 scenario. Aspects of climate change most likely to affect future distribution were considered as an increase in minimum and maximum Sea Surface Temperatures (SST). The methodology for the developed models is described in Annex VIII.

Baltic Sea: unlikely, high confidence
Greater North Sea: very likely, high confidence
Celtic Seas: very likely, high confidence
Bay of Biscay and the Iberian coast: very likely, high confidence
Mediterranean Sea: moderately likely, medium confidence
Black Sea: unlikely, low confidence

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): Ireland, in Galway Bay on the west coast of Ireland in 2001 and 2014 (see Radashevsky *et al*, 2019)

Response (8b):

Belgium: since 2011: Kerckhof & Faasse, 2014, however its presence is suspected since 2001, based on the re-examination of old material that was misidentified by Volckaert *et al.* (2003) as *Boccardiella ligerica* (Kerckhof & Faasse, 2014).
France: Atlantic coast since 2014: established: Spilmont *et al*., 2018/ English Channel 1999: Radashevsky *et al*, 2019.
Ireland: since 2001 (Radashevsky *et al.*, 2019).
Germany: 2016: Helgoland Island (Lackschewitz *et al*., 2017 in WGITMO, 2017) and backdated record from 2008, established (Kind & Kuhlenkamp, 2017; Radashevsky *et al.*, 2019).
Netherlands: since 2013 (Kerckhof & Faasse, 2014) established by 2017 (Wijnhoven *et al*., 2017)
Spain: Atlantic coast since 1996 (Martínez *et al*., 2006).
United Kingdom: first record in 2013 (Hatton & Pearce, 2013) backdated to 1998 by Radashevsky *et al.* (2019)

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): In addition to the countries where it is already established (Belgium**,** France, Germany, Ireland, Netherlands, Spain, United Kingdom**)** it may be established in Croatia, Denmark, Greece, Italy, Portugal, Slovenia.
The current maps indicate that in Malta and Cyprus the species will be constrained by high summer temperatures both now and in the future, and in Sweden by low salinity in the Baltic and by low winter temperatures along the Skagerrak. - See Annex VII, VIII

Response (9b): Belgium, France, Germany, Ireland, Netherlands, Spain, United Kingdom, Croatia, Denmark, Greece, Italy, Portugal, Slovenia and Sweden (an increase in winter temperatures will offer suitable climatic conditions along the Skagerrak).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: for details and references please see Q5.1

In summary: In sewage impacted sites in Argentina, *Boccardia proboscidea* creates epilithic biogenic reefs, smothers and displaces native species (most importantly native engineering species) and greatly alters the structure and function of the associated communities. The species is also known to bore into friable sedimentary rocks and soft sand/mudstone where, at high densities, it destroys the substrate. As a tube dweller in soft sediments, it can also create dense populations (examples from Australia, California and South Africa), which consolidate the sediments with their “tube mats”, impacts on the associated benthic communities however have not been reported.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: *Boccardia proboscidea* has been generally reported in low/medium densities in the RA area (see Qu. 5.2) with the exception of the island of Helgoland in the Greater North Sea (German Bight) and in North Tyneside, England, UK. In these locations the species is observed in high densities, boring into soft mudstone and concerns are expressed about potential erosion of the invaded habitat and the possible displacement of the native polychaete *Polydora ciliata* (Kind & Kuhlenkamp, 2017; Radashevsky *et al.*, 2019).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response: In Germany and the United Kingdom, see details above

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response: None

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided)”.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[2] and the provided key to pathways[3].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

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[2] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[3] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9

Pathway name:

TRANSPORT-STOWAWAY (ship/boat ballast water)

TRANSPORT-STOWAWAY (ship/boat hull fouling)

TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector) mariculture

a. TRANSPORT-STOWAWAY (ship/boat ballast water)

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	Unintentional	**CONFIDENCE**	medium

Response: In Argentina, Australia, the French English channel and the Netherlands, the discovery and proliferation of *Boccardia proboscidea* at locations close to ports and harbours has led to the hypothesis that ship-borne transport was the most likely pathway of introduction (Jaubet *et al*., 2018; Spilmont *et al*., 2018; Wijnhoven *et al*., 2017; Radashevsky *et al*., 2019).

On the other hand, on identifying and ranking introduced marine species found within Australian waters Hayes *et al*. (2005) ranked *B. proboscidea* as a low priority species to be introduced with ballast waters. The invasion potential of a species was expressed as the weighted sum of ship movements, and ballast discharge, from ‘infected’ bioregions to ‘uninfected’ bioregions.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	moderately likely	**CONFIDENCE**	high

Response: *Boccardia proboscidea* is capable of producing planktotrophic (free swimming) larvae, whose survivorship is favoured in cold-temperate waters (see Qu. 3.1, 3.10 and Annex VII). Each female can produce up to $10^2 – 10^3$ larvae per brood and up to 8 broods per year (for details on reproductive traits please see Qu. 3.9). It should be noted that the species achieves its highest densities (upwards of 10^5 individuals/m^2) in deteriorated and sheltered habitats (impacted by organic pollution) as for example in Australia, in Port Phillip Bay (Petch, 1989) which hosts the largest seaport in Australia.

The lowest estimates of the volumes of ballast water taken-up, transferred and discharged into world oceans each year are around 10 billion tonnes (Interwies & Khuchua, 2017), whereas just one cubic metre of ballast water may contain from 21 up to 50,000 zooplankton specimens (Locke *et al*., 1991, 1993; Gollasch, 1997) and a heavy bulk carrier can carry up to more than 130,000 tonnes of ballast water (GloBallast, 2009). While data on ballasts discharges are not available from EU organizations (EMSA, ECSA, ESPO) or other international relevant organisations, it is evident from the above information that the potential for sufficiently high numbers of *B. proboscidea* larvae to travel along this pathway is high.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	likely	**CONFIDENCE**	medium

Response: *Boccardia proboscidea* is capable of producing free-swimming, planktotrophic larvae, which spend 15-30 days in the water column before settling and whose survivorship is favoured at relatively low temperatures (12-17 °C) but is severely reduced at 24 °C (for more details and references, see Qu 3.1, 3.9, 3.10). Reproduction is clearly not an issue during ballast transport of larvae, however they are very likely to survive, as indicated by the detection and establishment of *B. proboscidea* populations in areas close to ports/harbors. Additionally, species of the Spionidae family are well known and often abundant constituents of both ballast waters and ballast sediments (e.g. Carlton, 1993; NRC, 1996; Gollasch, 1997).

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	After 2024 (with full compliance to the IMO D2): unlikely until 2024 (with current BWE): likely	**CONFIDENCE**	medium

Response: The International Maritime Organization (IMO) Ballast Water Management Convention (BWMC) entered into force in September 2017. It requires ships in international traffic to apply ballast water management measures, in particular:

- ballast water exchange in open seas, away from coastal areas (D-1 standard for an interim period)
- fulfil a certain discharge standard (D-2 standard according to the ship specific application schedule phased in up to 8 September 2024).
  D-2 standard requires the installation of a certified ballast water treatment device, which enables sterilization to avoid transfers of ballast water mediated species.

Ballast Water Exchange (BWE) is currently practiced and requires ships to exchange a minimum of 95% ballast water volume whenever possible at least 200 nautical miles (nm) from the nearest land and in water depths of at least 200 metres. When this is not possible, the BWE shall be conducted at least 50 nm from the nearest land and in waters at least 200 metres in depth (David *et al*., 2007 and BWMC Guideline 6). Even though BWE can reduce the concentration of live organisms in ballast by 80–95% (Ruiz & Reid 2007), its application has severe limitations, primarily dependant on shipping patterns of a port (e.g., shipping routes, length of voyages) and local specifics i.e., distance from nearest shore, water depth (David *et al*., 2007), particularly for EU Seas where these conditions are often not met. Also, organisms may still remain in the volume of ballast not exchanged, or BWE may not be possible due to weather conditions or other safety restrictions. The survival of zooplanktonic organisms (including *B. proboscidea*) is thus not unlikely when only BWE measure are implemented.

As a result, ballast water treatment has been deemed necessary, such that ships shall discharge (in relation to the organism size range of interest for *B. proboscidea*): less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension (IMO D-2 standard).

Ballast water treatment options include mechanical (filtration, separation), physical (heat treatment, ozone, UV light) and chemical methods (biocides). Efficiencies of various technologies utilised for ballast water treatment are reviewed in Tsolaki & Diamadopoulos (2009) and can vary with treatment method but the application of many combined methods (e.g. Filtration+UV or Hydroclone+chemical disinfectant) can decrease live zooplankton to undetectable levels, practically diminishing propagule pressure. As such, the survival of *B. proboscidea* larvae in ballast water with full implementation of the D-2 standard is considered unlikely.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: After September 2017, with the BWMC coming into effect and gradually being implemented, detection of larval stages in ballast water during Port State Control inspections may be possible. According to Resolution MEPC.252(67), if initial inspections of ballast water samples indicate non-compliance with the D-2 standard, detailed inpsections will be carried out. eDNA methodologies are rapidly becoming one of the fastest and most cost-efficient tools for the detection of NIS[4] in introduction water samples (Darling *et al.*, 2017; Borrell *et al.*, 2017; Koziol *et al.*, 2019) and have proven suitable for the detection of *B. proboscidea* larvae specifically in bilge water samples from vessels (Fletcher *et al.*, 2017). However, full implementation of the BWMC is not anticipated until 2024. Until then, the likelihood that *B. proboscidea* will enter the RA area undetected in ballast waters remains high.

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	After 2024 unlikely (IMO D2 is fully implemented) Until 2024 likely	**CONFIDENCE**	medium

Response: Regarding existing populations of the organism in the RA area (but also elsewhere in the invaded range), ballast water transport is considered one of the most likely pathways of introduction. Management measures implemented so far (i.e. BWE) have not proven adequate to prevent the introduction of this and other marine invasive species in EU marine waters. With the recent ratification of the BWMC (September 2017), compliance with the D2 standard is expected to greatly reduce the likelihood of secondary introductions of *B. proboscidea* in Europe with ballast water. However, this is not expected before 2024 and there may be difficulties in fully implementing it. For example, there are already some early reports of operational problems with the currently installed Ballast Water Management Systems, presumably due to

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[4] NIS: non-indigenous species, term used in the Marine Strategy Framework Directive, synonym of “alien species” as used in the framework of Regulation (EU) 1143/2014

poor installation and inadequate testing in the field (source: https://www.seatrade-maritime.com/news/asia/operating-problems-with-60-80-of-ballast-water-treatment-systems-intertanko/).

A concrete protocol for the verification of ballast water compliance monitoring devices has been proposed by IOC-UNESCO, ICES and ISO to IMO (IOC-UNESCO, ICES, ISO, 2019). This protocol builds on the method presented in documents PPR 6/4 and MEPC 74/4/11 (Denmark) (First et al., 2018) and suggests a protocol for verifying ballast water compliance monitoring devices using laboratory and shipboard tests. The intention is that the protocol can be the basis for the development of a standard for such devices, which may be used during commissioning testing, data gathering during the experience-building phase, compliance testing by port State control or self-monitoring.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

b. TRANSPORT-STOWAWAY (ship/boat hull fouling)

Qu. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	Unintentional	**CONFIDENCE**	medium

Response: It has been hypothesized but never documented. In most publications shipping traffic is reported as a potential pathway but the vector is not specified, it can be envisaged however that the organism can travel as part of a mature fouling assemblage, boring or hiding in/amongst attached bivalves, barnacles and other fouling organisms. The closely related, and also shell infesting species *Boccardia columbiana* Berkeley, 1927 was found in macrofouling of sea-chests of two commercial vessels in Canada (Frey *et al.*, 2014).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

RESPONSE	Moderately likely	**CONFIDENCE**	low

Response: For the reproductive biology of the species please see Qu 3.9. Considering the biological characteristics of the organism (ability to hide in crevices and burrows on/in bivalves, withstand desiccation and wide temperature range), it is considered possible that *B. proboscidea* can maintain a viable population within the fouling communities on ships hulls, sufficient for a new introduction.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	likely	**CONFIDENCE**	medium

Response: In a relatively wide range of temperatures, *B.proboscidea* is able to produce directly developing (adelphophagic) larvae that hatch at an advanced stage and settle close to the parent (see Qu 3.1, 3.9, 3.10). As such, it is possible that it can reproduce and maintain its fouling population during transport, and its cryptic lifestyle will protect it from the drag at high speeds of moving vessels.

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	Likely	**CONFIDENCE**	medium

Response: Fouling organisms such *as B. proboscidea* can be transported for weeks or months in or amongst other elements of the fouling assemblage (e.g. bivalve shells). Implementing practices to control and manage biofouling can greatly assist in reducing the risk of the transfer of invasive marine species.

While the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, 2001 (AFS Convention) addresses anti-fouling systems on ships, its focus is on the prevention of adverse impacts from the use of anti-fouling systems and the biocides they may contain, rather than preventing the transfer of invasive aquatic species.

Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species were adopted on 15 July 2011 [RESOLUTION MEPC.207(62)] The Biofouling Guidelines represent a decisive step towards reducing the transfer of invasive aquatic species by ships. Especially vessel cleaning during dry-docking in a shipyard generates a very low biosecurity risk because the debris is sent to local deposit and residue water from cleaning is collected (Bohn *et al*., 2016). Moreover, maintenance during dry-docking involves the re-application of anti-fouling paint, which seems to be efficient for up to 1-1.5 years – thereafter heavy fouling can start occurring (Sylvester *et al*., 2011; Frey *et al*., 2014).

On the other hand, in-water cleaning (IWC) of hulls (used as an additional tool, in-between dry-dock cleaning), especially without capturing the biofouling debris, might represent a higher risk of introducing NIS relative to land based cleaning in dry-docks with land based waste disposal because physical disturbance of the fouling communities may trigger the release of propagules or viable gametes (Hopkins & Forrest, 2008). The Resolution identifies this risk and consultations are in progress for more efficient management methods (e.g. Zabin *et al*., 2016). There has been a proliferation of new IWC technologies in the past decade (e.g. ttps://www.ecosubsea.com/, http://econetsaustralia.com/) that capture debris and render it non-viable through e.g. UV treatment, although such systems sometimes fail to contain all of the removed debris (for reviews see Zabin *et al*., 2016; Scianni & Georgiades, 2019).

Commercial ship-owners have a strong interest in having their vessels cleaned in order to decrease their fuel consumption but dry-docking frequency is determined by performance (fuel consumption below a certain threshold) and can range from 0.5-5 years (Bohn *et al*., 2016).

The suite of measures described above can prove effective against *B. proboscidea* and other fouling organisms, if fully implemented, although sea-chests would still remain higher risk areas and may require more frequent in-water treatment. However, anti-fouling practices are not legally required, and can be financially costly, making it likely that a number of vessels traveling between contaminated and uncontaminated marinas and ports will not have been treated, motivating a likely score for this question. Even though there does not appear to be any comprehensive analysis of the compliance levels to the MEPC 2011 biofouling guidelines, a considerable reduction in the arrival of high-risk vessels was observed in New Zealand, after biofouling management measures became mandatory (Hayes et al., 2019).

Although (as with physical hull cleaning), antifouling is not currently a legal requirement, there is potential that treatments with biocidal compounds may prove an effective method of controlling fouling and reduce the likelihood of introduction and spread.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	Very likely	**CONFIDENCE**	High

Response: The species is unlikely to be detected upon introduction, unless thorough inspections of hull fouling communities are carried out, which is currently not a routine practice. Even then, because, as part of the fouling community, it is most likely to be hidden within other fouling macro-organisms, the likelihood of detection via visual inspections is very low. Additionally, due to its similarity with other spionid species (see section A2), even upon detailed examination, the species may be confused with other species such as with *B. polybranchia* (Haswell, 1885), previously recorded in the English Channel (Dauvin et al. 2003).

In order to reach GES targets with reference to Descriptor D2, most EU states are already designing or implementing national/regional NIS-targeted monitoring programmes. Monitoring should focus on introduction hotspots (e.g. ports, marinas, aquaculture plots) and this will increase the detectability of *B. proboscidea* entering the RA area through hull fouling. Indeed one record of the burrowing worm *Boccardia proboscidea* was made from the regular monitoring programme in Orkney, UK (Kakkonen *et al*. 2019).

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	Moderately likely	**CONFIDENCE**	low

Response: It has been hypothesized but never documented. In most publications shipping traffic is reported as a potential pathway but the vector is not specified, it is however considered possible that the species can survive as part of the fouling community on ships hulls and release propagules upon arrival to new locations.

c. TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector) mariculture

Qu. 1.2c. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	Unintentional	**CONFIDENCE**	high

Response: *B. proboscidea* is a nuisance fouling species in aquaculture facilities thus aquaculture has been considered as the most probable pathway of its introduction in many parts of the invaded range. In fact, the species has been reported to heavily infest a shipment of oysters imported in Hawaii from Maine, USA (Bailey-Brock, 2000). Abalone farms were infested in New Zealand (Read, 2004), Tasmania (Lleonart, 2002) and South Africa (Boonzaaier *et al*., 2014). In South Africa, its introduction is almost certainly linked with shellfish transfers, either abalone from California or spat of the oyster *Crassostrea gigas* from Europe (Simon *et al*., 2009). In Belgium its presence may also be related to mariculture, since it was detected among the Pacific oyster *Crassostrea gigas (*Kerckhof & Faasse, 2014).

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: *B. proboscidea* on cultured molluscs can display high infestation rates, in the order of tens of individuals per shell (for rates of infestation, see Qu 5.9), shellfish imports however from countries outside the EU are not very common and prohibited in many cases, if not requiring strict quarantine procedures.

Available information in the peer reviewed literature suggests that bivalve culture in Europe is largely dependent on harvesting/collecting wild seed from nearby locations to aquaculture plots, bivalve seed from hatcheries to a smaller extent and, when necessary, imports of seed from other EU countries (Muehlbauer et al., 2014, Robert et al., 2013; Occhipinti-Ambrogi et al., 2016; Marchini et al., 2016). Small quantities of bivalves and other cultured molluscs may still be imported from non-EU countries (e.g. small quantities of oysters *C. gigas*, up to 3-4 tonnes per year, were directly imported from Japan and Korea into the Netherlands between 2004 and 2008 – Haydar & Wolff, 2011), but more extensive information on bivalve imports from countries outside the EU could not be found. The non-native abalone species *Haliotis discus hannai*, originally imported from Japan, is cultivated in Ireland (Hannon *et al*., 2013) and Spain (http://abalonbygma.com/en/abalone-exlusive-seafood/, see also Cook, 2014), where current production takes place in closed systems and relies on local hatcheries for seed. If more stock was imported from the native range, it would be subjected to the stringent controls of COUNCIL REGULATION (EC) No 708/2007 (see Qu.1.5c), such that this species is not considered to pose additional risks for new introductions of *B. proboscidea* into the RA area.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	Very likely	**CONFIDENCE**	high

Response: Spionids, or “mudworms“ have been regularly associated with shellfish consignments, as the shellfish themselves and the methods used to contain the shellfish during transport may actually enhance the likelihood of survival of contaminant species as well by providing moisture and protection from harsher conditions (Minchin, 2007). For example, the *Ostrea edulis* shipment that was imported to Hawaii in 2000 was heavily infested with adult *B. proboscidea*, whose burrows contained egg capsules with late-stage larvae, leading Bailey-Brock (2000) to conclude that reproduction had recently occurred, either before collection at the facility of origin or after placing the oysters in the recipient grow-out facility.

Qu. 1.5c. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	**moderately likely** (overall; local variations may apply)	**CONFIDENCE**	medium

Response: COUNCIL REGULATION (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed that minimise the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. It requires a permit procedure, involving risk assessment for the non-target species and a quarantine period for the translocated stock.

The bivalve *C. gigas* listed in Annex IV constitutes an exception and can be moved without any risk assessment or quarantine; however local/national legislation exists that can limit the translocation possibilities of species like *C. gigas*, e.g. see WG-AS & Gittenberger (2018) for the trilateral Wadden Sea area. Additionally, if the import region is a Natura2000 area regulations can be much stricter as they aim to protect the conservation objectives of the protected area first.

Other iniatives have produced codes of conduct for the transfer of bivalve seed/stock at the national/regional level, such as the ICES Code of Practice on the Introductions and Transfers of Marine Organisms 2005, the Code of practice for mussel seed movements (Wilson & Smith, 2008) Wales, etc.

The implementation of EC regulation 708/2007 (EC 2007) introduces a high biosecurity level for most bivalve transfers from areas outside the EU, that has already proven to be effective in preventing new introductions of marine alien species (Katsanevakis et al., 2013; Zenetos, 2019). However, the exemption of *C. gigas*, one of the main bivalve hosts of *B. proboscidea*, means that *C. gigas* consignments potentially infested with *B. proboscidea* would not be subjected to stringent control before being released into the wild, unless stricter national/regional regulations apply, thus increasing the risk of introduction of the species.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: Due to its size, cryptic nature and similarity with other spionid species (see section A2) and the frequent use of local (European) identification manuals, the species may be confused and remain unidentified/misidentified or go unreported (see also Qu. 1.6a, 1.6b). Moreover, *B. proboscidea* can deposit its eggs and larvae in the burrows created by other boring spionid pests (e.g. *Polydora hoplura*) or the calcareous tubes created by fouling spirorbid worms on the shells (Lleonart, 2002; Simon et al., 2009, 2010), such that eggs/larvae can easily go undetected by perfunctory visual inspections.

Qu. 1.7c. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	unlikely	**CONFIDENCE**	low

Response: The species may be introduced as pest, primarily on imported oysters *C. gigas* from the North East Pacific. Available literature suggests that shellfish imports from countries outside the EU are generally limited in the past couple of decades and well regulated. The risk of introduction is associated with a few species listed in Annex IV of Council Regulation (EC) No 708/2007 if stricter local/regional regulations are not in place (see Qu. 1.5d) and with illegal/unreported transfers. Even for the exempted species of Annex IV however (particularly *C. gigas* in Atlantic Europe, where establishment of *B. proboscidea* is more likely), the amount imported from outside the EU is at low enough levels to render this pathway unlikely.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	likely	**CONFIDENCE**	high

Response: Occurrence of *Boccardia proboscidea* at locations in the vicinity of ports and harbours has led to the hypothesis that vessels (either in ballasts or as fouling) is the most plausible pathway of its introduction. Management measures implemented so far (i.e. BWE) have not proven adequate to prevent its introduction in EU marine waters and this will partly continue until full implementation of the BWMC in 2024.

On the other hand, and based on its invasion history worldwide, aquaculture (i.e. contaminant on shellfish imported from outside the RA area) could be a very likely mode of its introduction but existing management measures scale down this probability significantly. Conclusively, vessels are a likely pathway of *B. proboscidea* introduction, mostly in the North East Atlantic, where prevailing temperatures favour the survival of planktotrophic larvae likely to be carried by ballast waters (see Risk of Establishment section).

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	likely	**CONFIDENCE**	high

Response: as in Qu. 1.9. Future climate change conditions are not anticipated to significantly change the likelihood of introduction of *B. proboscidea* through the above-mentioned pathways. Potential donor areas are not predicted to greatly expand/contract (see modelling results – global projected distribution – RCP4.5).

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[5] and the provided key to pathways[6].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name:

TRANSPORT-STOWAWAY (ship/boat ballast water)

TRANSPORT-STOWAWAY (ship/boat hull fouling)

TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector) mariculture

a. TRANSPORT-STOWAWAY (ship/boat ballast water)

Qu. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

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[5] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[6] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

RESPONSE	unintentional	**CONFIDENCE**	medium

On identifying and ranking introduced marine species found within Australian waters Hayes *et al*. (2005) ranked *B. proboscidea* as a low priority species to be introduced with ballast waters. The invasion potential of a species was expressed as the weighted sum of ship movements, and ballast discharge, from 'infected' bioregions to 'uninfected' bioregions.

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	After 2024 unlikely (with full compliance to the IMO D2) until 2024 likely	**CONFIDENCE**	medium

Response: The organism will enter into the environment during the de-ballasting process. See questions 1.3a and 1.5a for quantitative information on ballast volumes, propagule pressure and management practices that will affect entry of viable propagules.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: The organism has already entered Europe and it was detected when it had already established (Martínez *et al.,* 2006). In Belgium, its presence is suspected since 2001, based on the re-examination of old material that was misidentified by Volckaert *et al.* (2003) as *Boccardiella ligerica* (Kerckhof & Faasse, 2014).

The probability of observing the initial introduction event is minimal, particularly at the larval or early life stages but monitoring with settlement panels at introduction hotspots (Andersen *et al.*, 2014) and DNA barcoding in port water samples can increase the likelihood of early detection.

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	**CONFIDENCE**	medium

Response: For details on the reproductive biology of the species, see Qu. 3.9.

In summary, the reproductive period lasts from March to September in the northern hemisphere (Oyarzun, 2010) with multiple broods per year (up to 8). Considering that *B. proboscidea* reaches sexual maturity approximately 2.5 months after settlement (Simon & Booth, 2007) and maritime transport is carried out throughout the year, larvae picked up at locations with high *B. proboscidea* populations (occurring primarily in areas with mean summer temperatures higher or equal to 15 °C) have a high likelihood of arriving at a recipient area at a time appropriate for establishment – which is December to June, according to the information above.

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	likely	**CONFIDENCE**	high

Response: *Boccardia proboscidea* occupies a wide range of habitats, one or all of which are widely distributed in the RAA. It has proved very capable of colonising and dominating natural and man-made habitats

If ballast water exchange occurs in open seas rather than in coastal areas, transfer of planktonic larvae to suitable substrate will be hampered. If, however, untreated ballast water is released in ports, estuaries or other coastal areas, then establishment will be dependent on availability of suitable habitat. Considering a) the breadth of habitat that characterizes the species; b) the wide distribution of such habitats in the RAA and c) the documented records inside/near harbours (e.g. Spilmont *et al.*, 2018; Wijnhoven *et al*., 2017;

Radashevsky *et al*., 2019), there is a high likelihood that *B. proboscidea* can transfer to a suitable habitat after release with ballast water.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	After 2024 unlikely (with full compliance to the IMO D2, i.e. not until 2024) Until 2024 likely	**CONFIDENCE**	medium

Response: Regarding existing populations of the organism in the RA area (but also elsewhere in the invaded range), ballast water transport is considered one of the most likely pathways of introduction. Management measures implemented so far (i.e. BWE) have not proven adequate to prevent the introduction and entry of this and other marine invasive species in EU marine waters. With the recent ratification of the BWMC (September 2017) and full implementation anticipated until 2024, compliance with the D2 standard is expected to greatly reduce the likelihood of additional introductions of *B. proboscidea* in Europe with ballast water.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

b. TRANSPORT-STOWAWAY (ship/boat hull fouling)

Qu. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	medium

Response: *B. proboscidea* can transfer from a vessel's fouling assemblage through release of free-swimming larvae or as adult individuals, following the dislodgement of fouling material into the environment where the vessel is moored/berthed (MAF, 2011). Such vector has been hypothesized but never documented. In most publications shipping traffic is reported as a potential pathway but the vector is not specified, it can be envisaged however that the organism can travel as part of a mature fouling assemblage, boring or hiding in/amongst attached bivalves, barnacles and other fouling organisms. The closely related, and also shell infesting species *Boccardia columbiana* Berkeley, 1927 was found in macrofouling of sea-chests of two commercial vessels in Canada (Frey *et al*., 2014).

Global shipping and recreational boat travel take place between areas from which the species is known and ports, harbours and marinas within the RAA. The current concentration of records in and in close proximity to structures associated with recreational and commercial shipping suggest it is a potential vector of entry.

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	moderately likely	**CONFIDENCE**	low

Response: *B. proboscidea* can transfer from a vessel's fouling assemblage through release of free-swimming larvae or as adult individuals, following the dislodgement of fouling material into the environment where the vessel is moored/berthed (MAF, 2011). Both polychaetes and bivalves (in this case as potential hosts) are known to be translocated via hull fouling and remain viable (MAF, 2011 and references therein).

Global shipping and recreational boat travel take place between areas from which the species is known and ports, harbours and marinas within the RAA on a regular basis. The current concentration of records in and in close proximity to structures associated with recreational and commercial shipping suggest a potential source of propagules. *Boccardia* reproduces year-round and can produce up to eight broods throughout its reproductive period (Gibson 1997). Despite the reproductive output per individual not being particularly high per se, *B. proboscidea* regularly occurs in high densities both in the wild and in aquaculture systems, thus increasing the total number of propagules likely to be moved along the relevant pathways. Reports of population densities of tens to hundreds of thousands of individuals per m^2 and even higher are not uncommon in the literature (e.g. Oyarzun, 2010; Petch, 1995; see Impacts section for more details).

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response:- The organism has already entered Europe and it was detected when it had already established (Martínez *et al.*, 2006). In Belgium, its presence is suspected since 2001, based on the re-examination of old material that was misidentified by Volckaert *et al.* (2003) as *Boccardiella ligerica* (Kerckhof & Faasse, 2014).

Due to its size, cryptic nature and similarity with other spionid species (see section A2) and the frequent use of local (European) identification manuals, the species may be confused and remain unidentified/misidentified or go unreported (see also Qu. 1.6a, 1.6b). *Boccardia proboscidea* larvae released from ships fouling communities are highly unlikely to be detected early, considering the frequency of monitoring activities (especially in relation to larval stages). Furthermore, adults dislodged along with biofouling material will likely be hidden among/within other organisms, such as bivalves and barnacles. Adults can then deposit their eggs and larvae in the burrows created by other boring spionid pests (e.g. *Polydora hoplura*) or the calcareous tubes created by fouling spirorbid worms on the shells (Lleonart, 2002; Simon et al., 2009, 2010), such that eggs/larvae can easily go undetected by perfunctory visual inspections.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	**CONFIDENCE**	medium

Boccardia proboscidea reproduces for about 6 months per year (March to September in the northern hemisphere – see Qu. 3.9) and can produce up to eight broods throughout its reproductive period (Gibson 1997), thus it has high chances of arriving during the months of the year most appropriate to establishment.

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	likely	**CONFIDENCE**	medium

Response: *Boccardia proboscidea* occupies a wide range of habitats, one or all of which are widely distributed in the RAA. It has proved very capable of colonising and dominating natural and man-made habitats. Suitable habitats abound, especially in ports and marinas; indicatively at least two European records of *B. proboscidea* come from various substrates within harbours, i.e one scraped off Scapa Pier, Orkney (Kakkonen *et al*., 2018) and one from mud deposits under sandstone ledges in Staffin Harbour, Isle of Skye (Hatton & Pearce, 2013).

Considering a) the breadth of habitat that characterizes the species; b) the wide distribution of such habitats in the RAA and c) the documented records inside/near harbours (e.g. Spilmont *et al*., 2018; Wijnhoven *et al*., 2017; Radashevsky *et al*., 2019), there is a high likelihood that *B. proboscidea* can be deposited to a suitable habitat after release from fouling structures. In some cases, recreational vessels might moor in natural areas, adjacent to suitable natural substrate where propagules might be deposited.

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	moderately likely	**CONFIDENCE**	medium

Response: Although not formally documented, it remains plausible that *B. proboscidea* can be introduced and enter the RA area as a member of mature fouling communities on ships'/boats' hulls (see also Wijnhoven *et al*., 2017 for an assessment of likely pathways of introduction and spread), through the release of either larvae or adult individuals. As the BWMC is gradually implemented and until management measures for hull fouling become mandatory and/or widespread, this pathway will likely remain relevant for the introduction/entry of the species.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

c. **TRANSPORT-CONTAMINANT Contaminant on animals** (except parasites, species transported by host/vector) **mariculture**

Qu. 2.2c. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	medium

Response: as in Qu 1.2c

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: as in Qu. 1.3c and based on the well-regulated and limited imports of potential hosts from countries outside the EU.

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: as in Qu. 1.6c.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	**CONFIDENCE**	medium

Response: as in Qu. 2.5a

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very likely	**CONFIDENCE**	high

Response: If *B. proboscidea* infested bivalve seed/stock is relayed on cultivation plots without any prior management measure, as may be the case for *C. gigas* stock, the likelihood of transfer to other suitable habitats (the cultivation plots themselves are suitable habitats) is very high. These plots are often situated in coastal areas in close proximity to suitable natural habitat to-which individuals may spread.

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	unlikely	**CONFIDENCE**	low

Response: as in Qu. 1.7c and based on the well-regulated and limited imports of potential hosts from countries outside the EU. Unreported/illegal transfers are still a possibility, hence the low confidence rating.

End of pathway assessment.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	likely	**CONFIDENCE**	medium

Response: Occurrence of *Boccardia proboscidea* at locations in the vicinity of ports and harbours has led to the hypothesis that vessels (either in ballasts or as fouling) is the most plausible pathway of its introduction and entry. Management measures implemented so far (i.e. BWE) have not proven adequate to prevent its introduction in EU marine waters and this will partly continue until full implementation of the BWMC in 2024.

On the other hand, and based on its invasion history worldwide, aquaculture (i.e. contaminant on shellfish imported from outside the RA area) could be a very likely mode of its introduction but existing management measures scale down this probability significantly. Bilge waters as a vector of movement has been documented but the distances from the source areas are rather prohibitive. Conclusively, vessels are a likely pathway of *B. proboscidea* introduction and entry, mostly in the North East Atlantic, where prevailing temperatures favour the survival of planktotrophic larvae likely to be carried by ballast waters (see Risk of Establishment section).

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	likely	**CONFIDENCE**	medium

Response: as in Qu. 2.8. Future climate change conditions are not anticipated to significantly change the likelihood of introduction and entry of *B. proboscidea* through the above-mentioned pathways. Potential donor areas are not predicted to greatly expand/contract (see modelling results – global projected distribution – RCP4.5).

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: *Boccardia proboscidea* is a eurythermal and euryhaline species (Hartmann, 1940) with a cosmopolitan distribution in temperate seas. It is considered an introduced species in Australia, New Zealand, South Africa, Argentina and Hawaii (for details and references, see Qu. A5). Its presence in the Asian North-Pacific is of questionable origin (Sato-Okoshi *et al.*, 2000; Abe *et al.*, 2019a) and assumptions that it is native in Japan are not substantiated by concrete evidence (Radashevsky *et al.*, 2019).

The organism is already established in the RA area (Bay of Biscay and the Iberian coast, Celtic Seas, Greater North Sea) and further establishment in these MSFD marine subregions is considered very likely (see also Qu.A6, Qu.A7 and Annex VII, VIII). *B. proboscidea* has been recorded from locations with minimum yearly temperatures as low as 1.15 °C in northern China (Radashevsky *et al.*, 2019) and 2.6 °C in Japan (Imajima & Hartmann, 1964), it is more regularly encountered however in places where temperatures drop to 5-7 °C (Argentina, Canada) and in European waters between 3.7-6 °C (i.e. around the UK and the French, Belgian and Dutch North Sea coasts. Low winter temperatures in the Baltic (besides the salinity limitations – see below), as well as the Skagerrak and Kattegat, in the Greater North Sea, will hamper establishment in the region.

With regards to high temperature thresholds, the species can be found in Japan, at locations where the average temperature of the warmest month is in the region of ≈26.5 °C (species records from Abe *et al.*, 2019a – temperature values according to BIO-ORACLE data layers, Assis *et al.*, 2018, (http://www.bio-oracle.org/downloads-to-email.php). Additionally, it is known that in laboratory conditions, water temperatures of 24 °C and 28 °C severely reduce the survivorship of planktotrophic and adelphophagic larvae respectively (David & Simon, 2014 - see also Qu 3.9), while at 30 °C embryos do not develop at all (Oyarzun, 2010). The two types of larvae achieve survival optima at different temperatures (see also Qu. 3.10). Accordingly, high summer temperatures in the Levantine and large parts of the Ionian and the Central Mediterranean are expected to limit its distribution in the Mediterranean Sea to the relatively cooler regions of the basin (for details see Annexes VII & VIII).

With respect to salinity, in Australia, the species is established in conditions that range from brackish to fully marine (21 to 34.8 psu) (Coleman & Sinclair, 1996) but in laboratory experiments it has been shown to thrive at high salinities of up to 39-40 psu (Hillyard, 1979). Additionally, peak densities in Argentina were observed at salinities between 15-20 psu (Garaffo *et al.*, 2016), associated with increased organic matter conditions at untreated sewage outflows with high freshwater input. Thus, salinity would not be expected to pose limitations to survival and establishment at the range of values encountered in the Black Sea (SSS of 14-19 psu) but is very likely to become a prohibitive factor in most of the Baltic. In the Black Sea however, *B. proboscidea* would find itself at the edge of its physiological tolerance both in terms of temperature and salinity simultaneously, facing challenging conditions. The species distribution model predicts a low likelihood of establishment in this marine subregion (see Qu. 3.13 and Annex VII & VIII for more details).

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	ubiquitous	**CONFIDENCE**	high

Response: *Boccardia proboscidea* occupies a wide range of habitats, one or all of which are widely distributed in the RA area. Populations of *B. proboscidea* have been reported from different habitats including mudflats, sandy harbours, seagrass beds, among barnacles and mussels, in coralline algae, sandstone or sedimentary rocks, limestone reefs, artificial groynes, abrasion platforms, sewage outfalls and gastropod shells inhabited by hermit crabs (Hartman 1940; Woodwick 1963; Imajima & Hartman 1964; Petch 1989; Gibson 1997; Martinez, 2006; Kerckhof & Faasse, 2014; Jaubet *et al.*, 2015). The species has been found boring into sponge (David, 2015; Abe *et al.*, 2019a) and infesting bivalve (primarily oyster) and gastropod (abalone) shells (Simon *et al.*, 2010; Simon & Sato-Okoshi, 2015; Radashevsky *et al.*, 2019). It is most commonly encountered in the intertidal but has also been reported from subtidal locations in the wild at depths down to 100m (Imajima & Hartman, 1964), and in association with cultivated molluscs (Lleonart, 2002).

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	N/A	**CONFIDENCE**	high

Response: The organism does not require another species for critical stages in its life cycle.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	high

Response: In sewage impacted locations in Argentina, *B. proboscidea* outcompetes local intertidal species, and displaces the ecosystem engineering mussel *Brachidontes rodriguezii* as a structuring species within the intertidal due to competitive exclusion for space (Jaubet *et al.*, 2015). In non-impacted sites, *B. proboscidea* and *B. rodriguezii* coexist in patches of variable size (Jaubet *et al*., 2013). Again in Argentina, the invasion by *B. proboscidea* has been implicated in an apparent decline of its close relative, *Boccardia claparedei* (Kinberg, 1866) which occupies the same ecological niche (Radashevsky *et al*., 2013 – abstract) and the smothering of the barnacle *Balanus glandula* (Diez *et al*., 2011 – abstract). In European waters, the species has already established on rocky shores and intertidal areas among mussel beds (Martinez *et al*., 2006; Spilmont *et al*., 2018), thus further establishment is not expected to be prevented by competition with native species.

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	medium

Response: Polychaetes constitute an important part of the food chain and are eaten by a variety of infaunal as well as by epifaunal and pelagic species such as fish, molluscs and crustaceans (Hutchings, 1998). As an example, flatfish species have a documented preference for polychaetes in their diet, especially at the juvenile stage (Hinz *et al*., 2006) and *B. proboscidea* in particular is reported to dominate the diet of juvenile English sole *Parophrys vetulus* in its native range (Toole, 1980). Information on selective predation on *B. proboscidea* by native predators in the RA area could not be found, the species however is expected to play a similar role as elsewhere in the native and invaded range, where natural control by predation does not appear to be the critical factor for establishment.

Thus, based on its invasion history in the RA area, further establishment in suitable areas is not expected to be hampered by predation.

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	high

Response: The organism is already established in Europe, thus existing management practices related to introduction pathways/vectors could not prevent the entry into the environment of sufficient propagules for establishment.

Intensified monitoring for NIS, especially in hotspot areas, such ports and marinas has resulted in the recent detection of new (or previously missed) populations in Europe (e.g. Hatton & Pearce, 2013; Wijnhoven *et al*., 2017; Kakkonen *et al*., 2019). Considering, however, the detection history of the species with repeated misidentifications, the established populations in Europe and the length of the EU coastline that would need to be monitored, further establishment is considered very likely.

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	likely	**CONFIDENCE**	medium

Response: While Ballast Water Exchange (BWE) and Ballast Water Treatment (BWT) can reduce propagule pressure and, consequently, the rate of establishment (see Q1.6a for details), these management practices are not always possible or yet in effect. On the other hand, bivalve transportations for aquaculture purposes (which constitute a pathway of introduction and spread) offer suitable habitats to *B. proboscidea* in the form of the aquaculture plots themselves and, thus, facilitate establishment. Moreover, the exemption of *Crassostrea gigas* from Council Regulation (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture means that *C. gigas* spat/stocks can be moved without any risk assessment or quarantine (unless stricter local/regional regulations apply – see Qu. 1.5d concerning e.g. the Wadden Sea or Natura2000 areas), potentially exacerbating the risk of importing infested oysters. Coastal defenses, other man-made structures and reefs of escaped *Crassostrea gigas* will provide suitable habitat to facilitate the establishment of the species.

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	likely	**CONFIDENCE**	medium

Response: In protected conditions (i.e. aquaculture) eradication may be attempted, depending on the cultivated species, with freshwater or heated seawater immersion, the application of chemical agents or natural products, such as phyco-derived compounds, etc (Lleonart, 2002; Handlinger *et al*., 2004; Haupt *et al*., 2012; Simon *et al*., 2010). In such cases, the protection afforded by the burrow for adults/juveniles and by the capsule for the eggs and larvae can compromise the effectiveness of the treatment (compared with exposed worms/larvae in laboratory tanks) but this is dose/time/species specific.

Even though specific information on similar management efforts in the wild was not found, it is expected that the same biological properties of the organism would enhance its chances of survival following eradication campaigns in the wild. Additionally, the species has the ability to recover rapidly from disturbance, both at the small (Garaffo *et al*., 2016) and the large scale (Jaubet *et al*., 2011), presumably due to its breadth of habitat and reproductive strategy. In any case, eradication methods are not available for the planktonic larval stage, which, if already released, can travel long distances before settling on a suitable habitat.

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very likely	**CONFIDENCE**	high

Response:

Boccardia proboscidea lives for approximately 12 months, and reaches sexual maturity approximately 2.5 months after settlement (Simon & Booth, 2007). In the laboratory it reproduces year-round and can produce up to eight broods throughout its reproductive period (Gibson 1997). In the wild, the reproductive period last around 6 months, between March and September in the northern hemisphere (Oyarzun, 2010; Gibson 1997) and the respective spring and summer months in Argentina (Jaubet *et al*., 2015). Its reproduction is a well-documented case of poecilogony with two developmental modes (planktotrophic, adelphophagic), and three reproductive modes: Type I, egg capsules with only planktotrophic larvae; Type II, egg capsules with planktotrophic larvae and 15% nurse eggs (rare); and Type III, egg capsules with 90% nurse eggs and a mixture of planktotrophic larvae and adelphophagic larvae that feed on nurse eggs and hatch as advanced larvae or juveniles (Hartman 1940; Woodwick 1977; Gibson *et al*. 1999). Females can produce 30-60 capsules in a clutch, each of which contains an average of 60 larvae in Type I reproduction, and an average of 6 larvae plus nurse eggs in Type III reproduction (Gibson *et al.* 1999). Brooding time varies considerably with temperature; from 26 days at 12 °C to 9 days at 28 °C (David & Simon, 2014). Planktotrophs need to feed on the plankton after hatching (Pelagic Larval Duration PLD is ≈ 30 days for Type I and ≈ 15 days for Type III (Gibson, 1997)), whereas adelphophages eat nurse eggs and cannibalize small planktotroph siblings inside the capsule, hatching as juveniles that directly settle close to the adult (Gibson, 1997) or spend up to 4 days in the water column before settling (David, 2015). Optimal conditions for rearing in the lab are reported as 21 °C and 33 psu (David, 2015), while the range of temperatures under which spawning and development can be completed is very common along large parts of the RA area (Qu 3.1 and Annex VII).

Despite the reproductive output per individual not being particularly high per se, *B. proboscidea* regularly occurs in high densities both in the wild and in aquaculture systems, thus increasing the total number of propagules likely to be moved along the relevant pathways. Reports of population densities of tens to hundreds of thousands of individuals per m^2 and even higher are not uncommon in the literature (e.g. Oyarzun, 2010; Petch, 1995; see Impacts section for more details). Intensity of infestation of abalone in South Africa was up to ≈ 90 worms/shell in late winter, of which 1-8 were brooders and more than 50% were juveniles (Simon & Booth, 2007). Abalone farms rear tens of thousands of individuals per season, at high densities (Lleonart, 2002; Simon & Booth, 2007).

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Tolerance of a relatively wide range of temperatures and salinities and the habitat breadth of this species will very likely facilitate its establishment, particularly in Northern European waters. This will be further enhanced by the maternal control over the time of release of developing larvae from their capsules (Oyarzun and Strathmann, 2011), and the two types of larvae achieving survival optima at different temperatures. At relatively low temperatures (12-17 °C), females release larvae at an earlier stage of development, favouring the survivorship of planktotrophic larvae which manage to escape predation by their adelphophagous siblings (David & Simon, 2014). At higher temperatures, increased developmental rates result in shorter brooding times, increased adelphophagia and higher survivorship of directly developing larvae, potentially leading to strong local populations. In either case, the decision of when to rupture the capsules and release the larvae belongs to the mother, which, based on environmental cues, determines the optimal reproductive strategy for the population (Oyarzun and Strathmann, 2011; David, 2015).

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	likely	**CONFIDENCE**	medium

Response: Molecular studies with the 16S rRNA gene detected a single haplotype (haplotype A of Oyarzun, 2010, i.e. the second most widespread haplotype in the native range) for South African *B. proboscidea* populations (Simon *et al.*, 2009), which is shared among all south hemisphere populations (Radashevsky *et al.*, 2019). Radashevsky *et al.* (2019) also found a single 16S rDNA haplotype (haplotype K of Oyarzun, 2010, i.e. the most common and widespread haplotype in the native range) from UK and French populations. On the other hand, when examining the more variable cytochrome b mtDNA, which is considered more suitable for intraspecific studies (Simon *et al*., 2009; Williams *et al*., 2017), Simon *et al*. (2009) detected 7 haplotypes in South Africa. The most common of these was also detected in Vancouver, Washington and California, while the remaining 6 haplotypes were unique to South Africa. Based on these finding, Simon *et al*. (2009) suggested that *B. proboscidea* in South African abalone farms were ultimately derived from the same source, initially towards the central south coast abalone farms. The authors postulated that these populations were either subjected to more than one introduction or had the greatest haplotype diversity at the time of introduction.

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	moderately likely	**CONFIDENCE**	medium

Response: Multiple introductions have been hypothesized through shellfish transports in South Africa (Simon *et al*., 2009) and via ballast water transport for Australia (Radashevsky *et al*., 2019). The Hawaiian record of *B. proboscidea* on an oyster farm, caused by an *Ostrea edulis* shipment from Maine-USA (Bailey-Brock, 2000) has not been followed by any subsequent records in culture or in the wild (Radashevsky *et al*., 2019). Considering the volume of shellfish transports in the RA area, as well as the shipping traffic intensity, casual populations are likely to occur, particularly in areas of the Mediterranean and the Black Sea where environmental conditions do not favour establishment (see Qu.A6, Qu.A7 and Qu.3.1).

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very likely	**CONFIDENCE**	high

Response: Based on its invasion history in Atlantic Europe, the abiotic requirements, existence of similar conditions and availability of preferred habitats, the establishment of *Boccardia proboscidea* in the risk assessment area is considered very likely in the Celtic Seas, the Greater North Sea, the Bay of Biscay and the Iberian Coast. In the Baltic Sea, the species will be limited by low winter temperatures and low salinities and is considered unlikely to establish. This assessment is corroborated by the results of a newly developed species distribution model, presented in Annex VIII.

With respect to the Mediterranean Sea, high summer temperatures in the Levantine and in large parts of the Ionian and the Central Mediterranean are expected to limit *B. proboscidea*'s distribution in this marine region to its relatively cooler areas (see relevant maps Annexes VII & VIII). Even in these areas, maximum water temperatures above 24 °C are likely to reduce the survivorship of planktotrophic larvae and instead favour the development of adelphophagic larvae, for which the main pathway of introduction is anticipated to be shellfish transport. Thus, likelihood of establishment is predicted to be higher in areas of shellfish cultivation (oysters and mussels) and associated with high organic matter input and sheltered conditions. In the Black Sea, *B. proboscidea*'s salinity requirements are met but temperatures are similarly likely to favour directly developing larvae. Considering that bivalve cultivation in the Black Sea is not as developed/widespread as in the Mediterranean, introduction events are considered less likely to occur, resulting in an overall low probability of establishment. The species distribution model predicts low likelihood of occurrence in the Black Sea, presumably reflecting the fact that the species will find itself at the edge of its physiological tolerance both in terms of salinity and maximum temperature. At the same time, the low salinity tolerance is not likely to be picked up by the model due to limitations associated with the coarse resolution of global data layers (see Annex VIII for details), such that there is high uncertainty associated with this prediction.

Combining physiological tolerances and distribution modelling

very likely high confidence
Greater North Sea, Celtic Seas, Bay of Biscay and the Iberian coast

likely medium confidence
Mediterranean Sea

unlikely low confidence
Black Sea

unlikely high confidence
Baltic Sea

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.
With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very likely	**CONFIDENCE**	high

Response: Estimates in Annex VII refer to a maximum increase in seawater temperatures of 0.8 °C by 2065, according to the medium timeframe RCP 4.5 scenario. The SDM, presented in Annex VIII employed modelled future conditions for the 2070s under two different scenarios, RCP 2.6 and RCP 4.5.

Foreseeable climate change conditions are anticipated to lead to a slightly northward expansion of the species. In the Mediterranean Sea, increased maximum summer temperatures will restrict even more the areas with climatic conditions suitable for establishment to the north-eastern Aegean, small parts of the Adriatic and the cooler areas of the West Mediterranean. The development of localized populations is still possible under the circumstances mentioned above. Low likelihood of establishment is again predicted for the Black Sea, subject to the same limitations as mentioned above. An increase in minimum winter temperatures will offer suitable climatic conditions in parts of the Baltic Sea, low salinities however will continue to limit *B. proboscidea* in this marine region. Atlantic Europe will still offer highly suitable climatic conditions for the establishment of the species, for longer periods throughout the year at northern latitudes. A northward shift of the species is predicted by the model.

very likely high confidence
Greater North Sea, Celtic Seas, Bay of Biscay and the Iberian coast

moderately likely medium confidence
Mediterranean Sea

unlikely low confidence
Black Sea

unlikely high confidence
Baltic Sea

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic “spread” via release or escape (“jump-dispersal”), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

UNAIDED

RESPONSE	major	**CONFIDENCE**	high

Response:

Pathway Name: UNAIDED (Natural dispersal across borders from neighbouring countries, where the species has been introduced, (i.e. Spain, France, Belgium, Netherlands, UK, Ireland) by
a) planktotrophic larvae dispersed with oceanic currents
b) rafting on natural (e.g. logs, algae) debris

Boccardia proboscidea lives for approximately 12 months and reaches sexual maturity approximately 2.5 months after settlement (Simon & Booth, 2007). It can produce multiple broods throughout its 6 month reproductive period, which lasts between March and February in the northern hemisphere. It is capable of producing free-swimming, planktotrophic larvae, which spend 15-30 days in the water column before settling and whose survivorship is favoured at relatively low temperatures (12-17 °C) but is severely reduced at 24 °C (for more details and references, see Qu 3.1, 3.9, 3.10). The reproductive traits of the European populations have not been studied but it is expected that spring and summer temperatures prevalent throughout north-western European waters favour the development of naturally dispersing planktotrophic larvae, which is further supported by the expansion of the species along the French English channel and the Southern Bight of the North Sea near locations which constitute introduction hotpots, i.e. the port of Boulogne in France (Spilmont *et al*., 2018) and the Belgian and Dutch coast near ports and oyster culture plots (Kerckhof & Faasse, 2104; Wijhoven *et al*., 2017). The rate of spread due to natural dispersal cannot be teased apart from the rate of human-mediated spread (Qu. 4.2), especially since the species has very likely been overlooked, misidentified or not officially reported in the past in Europe (Radashevsky *et al.*, 2019; Kerckhof & Faasse, 2014; Hatton and Pearce, 2013) and it is very difficult to reconstruct an accurate timeline of spread, particularly without genetic data.

Rafting has been assumed as a means of dispersal of *B. proboscidea* from its original source populations in the south of Pacific USA northwards along the Pacific coast until Canada, as the species frequently establishes its tubes in logs, burrowing into the wood (Oyarzun, 2010). Debris such as drift-wood can travel great distances on ocean currents and would be capable of transporting and spreading reproductively viable worms within the RA area. Females store sperm such that adults boring into logs could continue reproducing, assuming they could find enough food resources to sustain them, with the ensuing larvae either directly settling next to the parent or freely dispersing. For information on propagule pressure, see Qu 3.9.

See also Qu 3.1, 3.9 and Annex VII.

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	major	**CONFIDENCE**	medium

Response: Pathway name:

TRANSPORT-STOWAWAY (ship/boat ballast water)

TRANSPORT-STOWAWAY (ship/boat hull fouling)

TRANSPORT-STOWAWAY (other – Ship/boat bilge water)

TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector) mariculture

According to the recent re-examination of older material and the tentative reconstruction of the invasion and spread timeline of the species (Radashevsky *et al*., 2019, Qu. A6, A8), within 5 years of the first European record in Spain in 1996 (Martinez *et al*., 2006), *B. proboscidea* was present in Brittany (1999), western

Ireland (2001), south-eastern UK (1998) and most likely Belgium by 2001, whereas Helgoland in Germany was already colonized by 2008 (Kind & Kuhlenkamp, 2017; Radashevsky *et al.*, 2019). While discoveries of *B. proboscidea* in relatively distant parts of northern Europe within a short period of time (late 1990s – early 2000s) may have arisen from several introduction incidents, it is considered likely that secondary spread through human assisted pathways has almost certainly played a role in its current distribution (Radashevsky *et al.*, 2019).

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name:

UNAIDED (Natural dispersal across borders from neighbouring countries)

TRANSPORT-STOWAWAY (ship/boat ballast water)

TRANSPORT-STOWAWAY (ship/boat hull fouling)

TRANSPORT-STOWAWAY (other – Ship/boat bilge water)

TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector) mariculture

Assuming eradication is highly unlikely throughout the European invaded range (see Qu 3.8), even if local populations were detected early and eradicated at specific locations, reinvasion and spread with natural dispersal from persistent populations is considered very likely.

a. TRANSPORT-STOWAWAY (ship/boat ballast water)

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	medium

Response. As in Qu.1.2.a

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	likely	**CONFIDENCE**	high

Response: For reproductive output and ship ballast volume & potential larval concentration, see Qu. 1.3a. With respect to spread of the organism within the EU, transshipment operations constitute the main maritime traffic that will act as the vector for spread. Important transshipment hubs are situated along the southern Mediterranean (serving the rest of the Mediterranean and the Black Sea) and the Le Havre-Hamburg range, serving the UK, the Baltic and Scandinavia (Notteboom *et al*., 2013). Considering that planktotrophic *B. proboscidea* larvae are favoured by water temperatures encountered throughout northern European Seas, it is considered likely that sufficient numbers can be transferred with ballast water along this pathway. See also Qu. 4.2. Higher water temperatures and the lack of established populations in the Mediterranean Sea, render this pathway of lesser importance for the spread of *B. proboscidea* in this marine region.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	medium

Response: As in Qu.1.4.a. Additionally, the smaller duration of short sea shipping routes between EU ports further increases the likelihood of survival within ballast waters, compared with international shipping routes.

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	unlikely (with full compliance to the IMO D2) likely (otherwise)	**CONFIDENCE**	medium

Response: See Q1.5a. BWE for EU short sea shipping routes is usually restricted to the second criterion of at least 50 nm from the nearest land and in waters at least 200 metres in depth in the Mediterranean Sea and is often not even feasible within these limits in northern European Seas (David *et al.*, 2007), such that ballast water exchange is not likely to be effective in preventing the spread of *B. proboscidea* (and other organisms potentially transferred in ballast water) within European Seas. Regarding the IMO D2 standard, compliance can practically diminish propagule pressure of zooplankton, but full implementation of the BWMC is not expected to happen before 2024.

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: as in Qu. 1.6a

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	likely	**CONFIDENCE**	high

Response: as in Qu. 2.6a

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	rapidly	**CONFIDENCE**	medium

Response: While it is not possible to assign separate rates of spread to the different pathways, discoveries of *B. proboscidea* in relatively distant parts of northern Europe within a short period of time (late 1990s – early 2000s) point to a relatively rapid rate of spread via human assisted pathways, of which ballast water transport is considered a likely vector within the RA area. See also Qu. 4.2

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

b. TRANSPORT-STOWAWAY (ship/boat hull fouling)

Qu. 4.3b. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	low

Response: as in Qu.1.2b

Qu. 4.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	moderately likely	**CONFIDENCE**	low

Response: Females of *Boccardia proboscidea* can produce 30-60 egg capsules in a clutch, each of which contains an average of 60 larvae in Type I reproduction, and an average of 6 larvae plus nurse eggs in Type III reproduction (Gibson et al. 1999); it is considered possible that *B. proboscidea* can spread within the RA area as a member of mature fouling communities on ships'/boats' hulls (see also Wijnhoven et al., 2017 for an assessment of likely pathways of introduction and spread), through the release of either larvae or adult individuals. – see also Qu 3.9 and 1.3b. Besides large vessels, particular mention is warranted of the role played by small leisure craft in the spread of marine NIS via hull fouling. Gittenberger *et al.* (2017), in a study focusing on non-native species in Dutch pleasure craft harbours between 2009-2016, demonstrated that, on average, 59% of all pleasure crafts studied (n=2055) in Dutch marine harbours had fouling on their hulls, with up to 25% of them carrying "heavy fouling" in the summer, i.e. abundant and often diverse fouling assemblages, covering >16% of the visible submerged surfaces. Even though *B. proboscidea* was not detected in these surveys, hull fouling communities included oysters *C. gigas* and barnacle species (Gittenberger *et al.*, 2017), that could serve as hosts of *B. proboscidea*, which would be overlooked unless fouling material is collected and carefully examined. Additionally, the closely related boring spionid *Polydora ciliata* was found on floating docks and settlement plates within the harbours.

Qu. 4.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	medium

Response: In a relatively wide range of temperatures, *B.proboscidea* is able to produce directly developing (adelphophagic) larvae that hatch at an advanced stage and settle close to the parent (see Qu 3.1, 3.9, 3.10). As such, it is possible that it can reproduce and maintain its fouling population during transport, and its cryptic lifestyle will protect it from the drag at high speeds of moving vessels.

Regarding intra-European voyages, and within EU marine regions in particular, recreational vessels are less likely to travel at high speeds and fouling species less likely to face as big changes in environmental conditions as those experienced during oceanic voyages (Ashton *et al*., 2006), such that their likelihood of survival increases. Moreover, leisure craft tend to remain at a single port for longer periods, which increases their chances of accumulating fouling species.

Qu. 4.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Hull cleaning is an often practiced method of defouling ship hulls and has the potential to physically remove *B. proboscidea,* which would in turn reduce the risk of spread Regarding large vessels, the suite of measures available for the management of biofouling (see Qu. 1.5b) can prove effective against *B. proboscidea* and other fouling organisms, if fully implemented, although sea-chests would still remain higher risk areas and may require more frequent in-water treatment. However, anti-fouling practices are not legally required, and can be financially costly, making it likely that a number of vessels traveling between contaminated and uncontaminated marinas and ports will not have been treated, motivating a high likelihood score for this question.

With respect to small leisure craft, Gittenberger *et al*. (2017) found that, although the majority (64%) of boat owners in the Netherlands haul their boats out of the water and clean them at least once a year, practices vary widely from harbor to harbor, with dry-docking/cleaning prevalence varying between 6% and 95%. This level of compliance still leaves plenty of opportunities for the spread of *B. proboscidea* within EU waters through fouling on recreational craft.

Qu. 4.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: see Q1.6b

Qu. 4.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	likely	**CONFIDENCE**	medium

Response: as in Qu. 2.3b, 2.6b

Qu. 4.9b. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	moderately rapidly	**CONFIDENCE**	medium

Response: Transport of *B. proboscidea* on ships hulls has been hypothesized but never documented. In most publications shipping traffic is reported as a potential pathway but the vector is not specified, it is however considered possible that the species can survive as part of the fouling community on ships hulls and release propagules upon arrival to new locations. It is not possible to assign separate rates of spread to the different pathways, but, considering the higher uncertainty associated with this pathway, its contribution to the overall potential for human assisted spread is assessed as relatively lower than ballast water (see Qu. 4.9a).

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

c. TRANSPORT-STOWAWAY (Bilge waters)

Qu. 4.3c. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	medium

Response: as in Qu 1.2c

Qu. 4.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway

- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	likely	**CONFIDENCE**	medium

Response: Response: It depends on a) the number of yachts and vessels arriving in a hub, b) theoretical distance and time to first discharge (assuming constant and linear travel) c) survival of the species and d) volume of bilge water.

See Qu 1.3c for bilge water volumes. An indication of the number of recreational vessels in the RA area is given by the European Boating Industry (2016), which estimates that over 6 million boats are kept in European waters while 4,500 marinas provide 1.75 million berths both inland and in coastal areas. Extrapolating from Gittenberger *et al*. (2017), approximately 30% of these vessels travel distances >100km from their home port. Assuming travel speeds of 5 knots (Fletcher *et al*., 2017), considerable distances can be travelled within the RA area within a matter of days, which significantly increases the likelihood that sufficient viable propagules of *B. proboscidea* can spread along this pathway from already established populations in the RA area.

Qu. 4.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	likely	**CONFIDENCE**	high

Response: It is related to propagule survival and typical distances travelled by yachts. The metabarcoding analysis of 23 bilge samples collected from yachts and motorboats operating commercially and recreationally in two boating hubs in New Zealand's South Island, lead to the identification of 5 NIS among which the polychaete *Boccardia proboscidea* (Fletcher *et al*., 2017). Considering the pelagic larval duration of *B. proboscidea* planktotrophic larvae (up to 30 days), the relatively short travel times of small vessels within the RA area and the tolerance of the organism to salinities down to approximately 15 psu, the likelihood of survival along this pathway is assessed as high.

Qu. 4.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	likely	**CONFIDENCE**	medium

Response: see Qu. 1.5c

Qu. 4.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	medium

Response: see Qu.1.6c

Qu. 4.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	likely	**CONFIDENCE**	high

Response: If viable propagules are discharged with untreated bilge water, they are likely to transfer to a suitable habitat or host, see Qu 2.3a, 2.3b, 2.6c.

Qu. 4.9c. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	moderately rapidly	**CONFIDENCE**	medium

Response: If present in a hub, which is not an uncommon occurrence for *B. proboscidea* (see e.g. Kakkonen *et al.*, 2019; Hatton & Pearce, 2013; Radashevsky *et al.*, 2019), the organism can easily spread to the next destination of a leisure craft or other type of small vessel. Considering the much lower volumes of bilge water transported (compared to ballast water), and the generally smaller distances covered by recreational boats compared to large vessels, the contribution of this pathway to the overall potential for human assisted spread is assessed as relatively lower than ballast water (see Qu. 4.9a)

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

d. TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector) mariculture

Qu. 4.3d. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	Unintentional	**CONFIDENCE**	high

Response: All countries along the European Atlantic and the Mediterranean coasts involved in the cultivation of bivalves are currently conducting transfer activities (Muehlbauer *et al.,* 2014; Occhipinti-Ambrogi *et al.*, 2016; Marchini *et al.*, 2014; Rodrigues *et al.*, 2015). These activities include transfers at all life stages, from field sites to wild fishery sites or from field to culture sites, from shore to onshore facilities or from nearshore wild bottom beds to offshore hanging cultivation devices (Muehlbauer *et al.*, 2014). Based on its invasion history in South Africa, where *B. proboscidea* worms were spread among farms in South Africa primarily through the transport of infested abalone (Simon *et al.,* 2009), and its well known history of bivalve infestation (particularly oysters – Simon & Sato-Okoshi, 2015), the species is considered likely to spread within the RA area as an aquaculture pest. Particularly noteworthy is the danger of spread through this pathway to the Mediterranean Sea, where the species is not currently present/established (e.g. both Spain and France transfer bivalve seed/stock between Atlantic and Mediterranean cultivations sites - Muehlbauer *et al.,* 2014). In fact, the discovery of the species in an oyster, purportedly originating in Leucate Lagoon, France (Radshevsky *et al*., 2019) raises concerns about the role of aquaculture on the potential spread of *B. proboscidea*.

Qu. 4.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	likely to very likely	**CONFIDENCE**	medium

Response: *B. proboscidea* on cultured mollusks can display high infestation rates, in the order of tens of individuals per shell (for rates of infestation, see Qu 5.9). The species has already been recorded among oysters (Wijnhoven *et al.*, 2017) and intertidal mussel reefs (Martinez *et al.*, 2006; Spilmont *et al.*, 2018) as an interstitial organism in small to medium densities (See Qu. 5.2), which are considered likely to act as sources of infestation. Given the large volume of shellfish transfers within the RA area, the potential for sufficient individuals of *B. proboscidea* spreading along this pathway is high.

for more details see also Qu.1.3d

Qu. 4.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Spionids, or „mudworms“, have been regularly associated with shellfish consignments, as the shellfish themselves and the methods used to contain them during transport may actually enhance the likelihood of survival of contaminant species as well by providing moisture and protection from harsher conditions (Minchin, 2007). For example, the *Ostrea edulis* shipment that was imported to Hawaii in 2000 was heavily infested with adult *B. proboscidea,* whose burrows contained egg capsules with late-stage larvae, leading Bailey-Brock (2000) to conclude that reproduction had recently occurred, either before collection at the facility of origin or after placing the oysters in the recipient grow-out facility.

Qu. 4.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	likely (overall score; local variations may apply)	**CONFIDENCE**	high

Response: Response: COUNCIL REGULATION (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed that minimise the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. It requires a permit procedure, involving risk assessment for the non-target species and a quarantine period for the translocated stock. Importantly, in relation to spread within the RA area, the regulation does not apply to movements of locally absent species within the Member States "except for cases where, on the basis of scientific advice, there are grounds for foreseeing environmental threats due to the translocation, Art. 2 para. 2."

Additionally, the bivalve *C. gigas* listed in Annex IV, which is one of the main bivalve hosts of *B. proboscidea* both in cultivation and in the wild, constitutes an exception and can be moved without any risk assessment or quarantine. However local/national legislation exists that can limit the translocation possibilities of species like *C. gigas*, e.g. see WG-AS & Gittenberger (2018) for the trilateral Wadden Sea area. Moreover, if the import region is a Natura2000 area regulations can be much stricter as they aim to protect the conservation objectives of the proteceted area first.

Other iniatives have produced codes of conduct for the transfer of bivalve seed/stock at the national/regional level, such as the ICES Code of Practice on the Introductions and Transfers of Marine Organisms 2005, the Code of practice for mussel seed movements (Wilson & Smith, 2008) Wales, etc. In general, restrictions on transfers based on the risk associated with the source areas is an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk NIS are available; for *B. proboscidea*, difficulties in detection and identification can hamper such efforts.

Qu. 4.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: Eggs/larvae can easily go undetected by perfunctory visual inspections. Mature individuals can be misidentified.

Qu. 4.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very likely	**CONFIDENCE**	high

Response: If *B. proboscidea* infested bivalve seed/stock is relayed on cultivation plots without any prior management measure, the likelihood of transfer to other suitable habitats (the cultivation plots themselves are suitable habitats) is very high. These plots are often situated in coastal areas in close proximity to suitable natural habitat to-which individuals may spread. Regarding abalone cultivation practices, these species are grown in land-based facilities, from which *B. proboscidea* can escape through the farms' outflow waters, as it happened in South Africa (David, 2014 and references therein).

Qu. 4.9d. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	moderately rapidly	**CONFIDENCE**	medium

Response: see Qu. 4.9a. Bivalve transfers are a likely mechanism of spread in the RA area, although, considering the degree of regulation of the industry and the fact that in many cases transfers are predominantly conducted within Member States, spread to distant locations through this pathway may be less important than spread through ship-mediated pathways.

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Pathway Name: **UNAIDED**

Qu. 4.3e. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: By definition unintentional

Qu. 4.4e. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	likely	**CONFIDENCE**	high

Response: Females of *Boccardia proboscidea* can produce 30-60 egg capsules in a clutch, each of which contains an average of 60 larvae in Type I reproduction, and an average of 6 larvae plus nurse eggs in Type III reproduction (Gibson et al. 1999) – see also Qu 3.9. Despite the reproductive output per individual not being particularly high per se, and the current densities of *B. proboscidea* in the RA area not being very high, the relatively wide spread of the species in the RA area, particularly near introduction hotspots, indicates that the number of individuals (be it free swimming larvae or rafting adults) spreading unaided has been sufficient to originate viable populations in new locations.

Qu. 4.5e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: The species is very likely to reproduce, producing both planktotrophic and adelphophagic larvae, whose likelihood of development and survival depends upon temperature, as depicted in the maps of Qu 3.1 and the Annex. Free-swimming larvae, as well as adults boring into logs are perfectly capable of surviving in the climatic conditions present throughout large parts of the RA area, while natural dispersal is expected to be more important in Atlantic Europe compared with the Mediterranean and Black Seas. Females store sperm such that adults boring into logs could continue reproducing, assuming they could find enough food resources to sustain them, with the ensuing larvae either directly settling next to the parent or freely dispersing.

Qu. 4.6e. How likely is the organism to survive existing management practices during spread?

RESPONSE	very likely	**CONFIDENCE**	high

Response: No management practices are in place concerning natural dispersal that can affect the organism's ability to survive along this pathway

Qu. 4.7e. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	likely	**CONFIDENCE**	high

Response: The species has very likely been overlooked, misidentified or not officially reported in the past in Europe (Hatton & Pearce, 2013; Kerckhof & Faasse, 2014; Radashevsky *et al.*, 2019). *Boccardia proboscidea* is easily missed by many routine survey methods because its preference for intertidal firm or hard substrata excludes it from most routine grab and core samples (Radashevsky *et al.*, 2019). Intertidal rocky shore or artificial hard substrates have not been the focus of regular monitoring schemes (Spilmont *et al.*, 2018; Kerckhof & Faasse, 2014); additionally, the use of European identification keys that may not include non-native species can lead to misidentifications.

Qu. 4.8e. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very likely	**CONFIDENCE**	high

Response: During natural dispersal, organisms usually arrive and settle in suitable habitats or move on. *Boccardia proboscidea* larvae settle on a variety of habitats, including sand, broken mollusk shells, alive mollusks (Gibson, 1997; Simon and Booth, 2007; Oyarzun, 2010); settlement habitats are widely available throughout the RA. Similarly, individuals traveling on floating debris can easily find settlement habitats both on hard and soft substrates once the rafting material reaches the shore.

Qu. 4.9e. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	moderately rapidly	**CONFIDENCE**	medium

Natural dispersal has almost certainly played (and will keep playing) an important role in the spread of *B. proboscidea* within the RA area, particularly in Atlantic Europe, however it is rather unlikely that it is the mechanism responsible for the appearance of the species at locations as distant as northern Spain, western Ireland, south-eastern UK and Belgium within a period of approximately 5 years (see QU. A6, A8). While larval dispersal will proceed at different rates depending on local/regional hydrodynamic conditions and topography, on average a moderate rate of spread is expected. In the Mediterranean Sea, where high temperatures will favour the survival of directly developing larvae, natural spread may be even slower and of lesser importance at the regional scale. Directly developing larvae (also called adelphophagic) hatch as juveniles that directly settle close to the adult (Gibson, 1997) or spend up to 4 days in the water column before settling (David, 2015), thus having reduced natural dispersal by currents.

End of pathway assessment

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very difficult	**CONFIDENCE**	high

Response:

Naturally dispersing organisms are very difficult to contain. However, early detection, rapid response and control in aquaculture sites is feasible (Grosholz & Ruiz, 2002), if the species is correctly identified and restrictions based on the risk associated with the source areas are rapidly adopted by the industry. The current legal instruments and levels of implementation of voluntary measures are not sufficient to ensure containment of the organism, when transferred by ballast water (but this can change with full implementation of the D-2 Standard), fouling assemblages on ships' hulls, or bilge water. See also responses to Qu 4.6a-d.

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	rapidly	**CONFIDENCE**	high

Response: Unaided dispersal and multiple pathways of human-aided spread create a considerable potential for rapid spread, in the order of 10^3 km within 5 years. Among the ship-mediated pathways, ballast transport is considered more likely to have been responsible for the already manifested rapid rate of spread along Atlantic Europe and will continue to play the same role until the BWMC is fully implemented. Hull fouling and bilge waters, especially of leisure and other small craft may have also contributed to the current spread of *B. proboscidea* in northern European waters; bilge waters in particular is a vector that has been overlooked until recently and it appears to be able to transport viable propagules of the species in the relatively short duration of intra-European journeys. Finally, bivalve transfers are another likely mechanism of spread in the RA area, especially within Member States but potentially also between marine regions/subregions. Particular attention is needed when transferring oyster consignments between Atlantic Europe and the Mediterranean.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	rapidly	**CONFIDENCE**	high

Response: Foreseeable climate change conditions are anticipated to lead to a slightly northward expansion of the species (see model results in ANNEX VIII). Natural dispersal may be slightly accelerated, as Atlantic Europe will offer highly suitable climatic conditions for the establishment of the species for longer periods throughout the year at northern latitudes (but still within the 12-17 °C bracket optimal for planktotrophic larvae), increasing brood frequency and production. In the Mediterranean Sea on the contrary, elevated maximum temperatures will make bivalve transport the dominant potential spread mechanism for *B.*

proboscidea and may further enhance its importance. For example, heat waves can cause mass mortality of aquaculture bivalves, leading to increased shellfish transfers to replete the stocks (Rodrigues et al., 2015).

Finally, higher frequency and severity of storms and hurricanes can increase the amount of large floating debris, further enhancing natural dispersal. Higher frequency of inclement weather may also lead to higher port residence times for vessels, increasing the likelihood of development of fouling communities and of releasing propagules (Galil et al., 2019).

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	major	**CONFIDENCE**	high

Comment: *Boccardia proboscidea* is considered an introduced species in Australia, New Zealand, South Africa, Argentina, Hawaii and of uncertain origin in Japan, China, Korea (see Qu A5), it is only in Argentina however that severe environmental impacts have been documented.

At sewage impacted sites, *B. proboscidea* displaces the native ecosystem engineering mussel *Brachidontes rodriguezii* from the rocky intertidal, forming solid epilithic biogenic structures termed “reefs” (Jaubet *et al*., 2011, 2013). At sites heavily impacted by untreated sewage effluent, these reefs covered up to 70-100% of the substrates from 50 to 1200 m south of the sewage outfall (Jaubet *et al*., 2013; Elias *et al*., 2014), with worm densities upwards of 10^6 individuals/m^2 (Garaffo *et al*., 2012). The dominance of *B. proboscidea* significantly reduced the species as well as the functional richness and diversity of the rocky intertidal flora and fauna (Elias *et al*., 2014; Garaffo *et al*., 2018). The explosive population increase of *B. proboscidea* and the competitive exclusion of *B. rodriguezii* was attributed to a combination of increased organic input with the weakened condition of the native mussel due to sewage contamination. In Mar del Plata, Argentina, *B. proboscidea* is considered primarily a boring species, boring into coastal abrasion platforms and building massive aggregations of tubes over them, and secondarily a tube-dwelling species, where increased sedimentation in areas of sewage discharge is forcing the construction of tubes (Jaubet *et al.*, 2014). Major impacts on rocky intertidal habitats have also been documented in northern Patagonia, where *B. proboscidea* populations bore into friable sedimentary rocks where they destroy the substrate (see images below, depicting the scale and intensity of the boring activity and ensuing damage) and greatly alter the native communities (Radashevsky *et al.*, 2013). The species is now distributed along most of the Argentinean coastline (Jaubet *et al.*, 2018), where it has become a dominant and permanent fixture in the successional dynamics of the intertidal benthic communities, both on hard and on soft substrates (Llanos *et al.*, 2019), aided by its large capacity to rapidly recover after disturbance (Becherucci et al., 2016). Additionally, *B. proboscidea* in high densities on man-made and natural hard substrates smothered populations of native [to Argentina] mussels *Brachidontes* spp. and of the alien barnacle *Balanus glandula* (Diez *et al.*, 2011).

Image: Rock surfaces heavily infested with *B. proboscidea* in Chubut province, Argentina, depicting the structural damage to the habitat. Unpublished photographs used with permission by V. Radashevsky.

Outside Argentina, structural impacts of *B. proboscidea* on soft-sediment habitats have also been observed in South Africa at the outflow path of an abalone farm (David, 2015) and in Australia, Port Phillip Bay, in areas of secondary treated sewage discharge (Petch, 1989). In both cases, high densities (e.g. Petch, 1989 reported densities of 350000 ind./m^2) of the organism from "tube mats" and consolidate the sediments, impacts on the associated benthic communities however were not reported, neither was the formation of reefs, similar to those that developed in Argentina (Carol Simon, pers. comm, May 2019). In South Africa, the species was initially associated with onshore abalone farms (Simon *et al*, 2007; 2009; 2010) and took several years to establish in the wild (David, 2015), primarily around the farms' outflow paths. The lack of severe impacts was attributed to the fact that abalone farms are usually in high energy areas, where high wave action quickly distributes effluents (Carol Simon, pers. comm, May 2019).

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	moderate	**CONFIDENCE**	low

Comment: In the Risk Assessment area, *Boccardia proboscidea* is relatively widespread along the North East Atlantic (see Qu.A6, Qu.A8) but, to date, only low to moderately high densities have been recorded, with the exception of the island of Helgoland and one location in the UK.
Spain: The highest densities of the species were obtained in the spring of 1997, with more than 5000 individuals/m^2, next to a sewage outfall and on the calcareous rhodophyte *Corallina elongata* and alongside the bivalves *Mytilaster minimus* (Poli, 1795) and *Mytilus galloprovincialis* (Martinez *et al*., 2006). This observation confirms that, under conditions of organic enrichment, *B. proboscidea* can start proliferating and dominating intertidal communities in the RA area.
France: English Channel (Opal coast) highest density of 263 ind./m^2 (Spilmont *et al*., 2018) on intertidal mussel reefs.
Belgium: Densities varied between 100 ind./m^2 (Koksijde 2012) and 1250 ind./m^2 (Oostende 2013) (Kerckhof & Faasse, 2014).
Germany: On the island of Helgoland in the German Bight *Boccardia proboscidea* is observed in high densities, boring into soft mudstone (in the order of at least 20000 ind/m^2, roughly estimated from Kind & Kuhlenkamp, 2017). Concerns are expressed about potential erosion of the invaded habitat and the possible displacement of the native polychaete *Polydora ciliata* (Kind & Kuhlenkamp, 2017; Radashevsky *et al*., 2019). Increased erosion of the invaded abrasion platforms may affect the fucoid and mytilid communities developing on them (Bartch & Tittley, 2004; Kuhlenkamp *et al*., 2011).

United Kingdom: another dense population has been observed in Tyneside, Northeast England, however no quantitative data are available for this location. Worms were in silty tubes in algal mats on sandstone and also in crevices and boring into sandstone (Radashevsky *et al*., 2019 and personal communication).

Regarding other European locations, the species has been recorded from hard substrata, natural (intertidal rocky shores, boring into rocks) and artificial (piers, groynes, etc), intertidal soft sediment on hard substrata (Radashevsky *et al*., 2019), interstitially among oysters (Wijnhoven *et al*., 2017), and turf but no additional reports of densities or demonstrated impacts were found.

Based on reported values elsewhere in the world, it is estimated that densities in the order of 10^4-10^5 and above may start seriously affecting local biodiversity. Currently, this seems to be the case in Helgoland, Germany, where there are signs of possible displacement of *P. ciliata* by high densities of *B. proboscidea*. Based on qualitative observations, it is possible that community impacts as well as structural impacts on soft rock substrates have also occurred in the UK but there is currently no sufficient evidence to quantify their extent and severity. At organically enriched locations, *B. proboscidea* at moderate densities (e.g. San Sebastian, Spain) may be in competition for space with native mytilids, oysters and barnacles and possibly structuring algae, however no negative effects have been studied or documented to date. *Mytilaster minimus*, a species that rarely occurs outside the Mediterranean (Morton & Puljas, 2017), and is already under competitive pressure by the native *Mytilus galloprovincialis* and the alien *Brachidontes pharaonis* (Cinar *et al*., 2017 and references therein), may be particularly vulnerable to population increases of *B. proboscidea*.

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	major	**CONFIDENCE**	low

Comment: This depends entirely on the densities the species attains in the RA and it is rather difficult to predict with any certainty, especially because incidents of population explosions are linked with localized sources of organic enrichment. Areas where untreated sewage effluents (and even treated wastewater, see Qu 5.1) are discharged into the sea, as well as the immediate vicinity of aquaculture facilities could provide such hot-spots for *B. proboscidea* proliferation. Intertidal mussel and oyster beds/reefs are important habitats, both ecologically and commercially, and are potentially at risk as they constitute hotspots of introduction. The fact that, more than 20 years after the first record (1996, documented in Martinez *et al*., 2006), high population densities have been reported in only two locations, could be due to misidentification with other spionid species or understudied habitats. However, as the species continues spreading in European Seas, particularly the North East Atlantic, and awareness among experts is rising, the likelihood of population explosions occurring and becoming detected is bound to increase (e.g. the recent case of Helgoland, where the species has been present since at least 2008 but high densities and impacts were only reported after 2017). Additionally, under foreseeable climate change conditions, increased frequency and intensity of storms associated with increased coastal erosion and terrigenous inputs, has the potential to create conditions suitable for spionid outbreaks both on cultured and wild populations of bivalves, i.e. high siltation rates and organic matter inputs that can reduce the fitness of the bivalves and promote both infestations and tube-building and smothering by the worms (Ogburn *et al*., 2007; Clements *et al*., 2017 and references therein; Jaubet *et al*., 2018). In this case, population declines of native intertidal species may be evidenced, associated with changes in community structure, as well as structural impacts on both hard and soft substrates. Importantly, the species' boring activity has the potential to permanently alter soft rock habitats in the RA area, similarly to what was observed in Patagonia, causing irreversible damage to the substrate with a very high density of burrows, and potentially enhancing erosion processes. See also Qu 5.4-5.5.

The potential future impact on native biodiversity is therefore assessed as major.

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	moderate	**CONFIDENCE**	low

Comment:

At present, impacts on the conservation value of habitats and species in the RA area have not been quantified, it should be noted however that the rocky littoral of Helgoland is a marine protected area since 1981 and a reference site for ecological comparisons of European rocky shore biotopes (Reichert & Buchholz, 2006). The abrasion platforms of the island, assigned to EUNIS Biotope A4.23 Communities on soft circalittoral rock, are already invaded by high densities of *B. proboscidea*, which is displacing the native spionid *P. ciliata* (see Qu 5.2) and are under an increased risk of bioerosion due to the larger size of the invader (up to 45mm long compared to 1-3mm for the native).

See potentially threatened habitats in the following question

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	major	**CONFIDENCE**	low

Comment: The following biotopes and habitats are suitable for colonization by *Boccardia proboscidea* and may be endangered:

- Mussel beds on infralittoral rock are part of the wider Reef NATURA-1170 habitat type (Annex I of the Habitats Directive). The habitat is also part of the Sublittoral rocky seabeds and kelp forests (code 11.24), listed as endangered in the Resolution no. 4 of the Council of Bern Convention (1996) (Salomidi *et al.*, 2012).
- In addition to A4.23 described in the previous question, other biotopes and habitats on infralittoral and circalittoral rock (under EUNIS code A.3 and A.4 respectively), and especially reefs made from soft rock (e.g. chalk reefs along the SE English coast) may be at particular risk of erosion and alteration of the associated communities. Vertical cliffs and gently-sloping intertidal platforms made from chalk support a range of micro-habitats of biological importance and unique faunal communities (OSPAR 2008). Such coastal exposures of chalk are rare in Europe; littoral chalk communities are on the OSPAR List of Threatened and/or Declining Species and Habitats (Fletcher et al., 2012).
- Mussel beds on circalittoral rock (EUNIS A4.24)
- Sublittoral mussel beds on sediment (EUNIS A5.62). Within the Habitats Directive, this biotope can be protected as Reefs (habitat type 1170).
- *Ostrea edulis* beds on shallow sublittoral muddy mixed sediment are part of the wider Reef NATURA-1170 habitat type (Annex I of the Habitats Directive). They are included in the European Red List of Habitats as Critically Endangered (EU 2016).
- Infralittoral mussel beds are of conservation concern (Near Threatened to Critically Endangered) across the regional seas.
- In the Mediterranean Sea, both mussel beds A5.6v (*M. galloprovinciallis*) and native oyster beds A5.6y (*Ostrea edulis*) are included in the European Red List of Habitats as Vulnerable (EU, 2016).
- In the Black Sea, Pontic *Ostrea edulis* biogenic reefs on mixed and rocky sea bottom qualify for NATURA 2000 habitat type 1170 (Reefs).

Moderate impacts may be expected in mussel and oyster habitats due to the primarily interstitial lifestyle of *B. proboscidea* in the former and its limited boring activity on the latter (see Qu 5.1, 5.2 and 5.9). On the other hand, littoral soft rock (chalk) habitats and their associated communities may be at higher risk of severe damage if infested by high densities of *B. proboscidea*, hence the major score.

Other endangered habitats under the Habitats Directive include: Estuaries-1130, Coastal lagoons-1150, Large shallow inlets-1160 (Natural England 2016).

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

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RESPONSE	moderate	**CONFIDENCE**	low

Comment: *Boccardia proboscidea* infests molluscs (bivalves and gastropods) both in aquaculture systems and in the wild and can thus affect food provisioning services. In Argentina, the native mussel *Brachidontes rodriguezii*, which is displaced by *B. proboscidea*, is reportedly subjected to artisanal and recreational hand harvesting (Carranza *et al*., 2009), however, no impacts on related provisioning services were reported in the series of articles documenting the ecological impact of *B. proboscidea* on *B. rodriguezii* communities (Jaubet *et al*., 2011, 2013, 2015; Garaffo *et al*., 2012, 2016, 2018; Elias *et al*., 2014, etc.). This could be attributed to the fact that population explosions of the invasive polychaete occurred at sewage impacted sites that would be unsuitable for mussel harvesting or to the fact that artisanal harvesting of the mussel may not be a widespread activity of high importance. Similarly, infestations of wild oyster populations in Asia and Australia have not been accompanied by ecosystem services impact reports (e.g. Sato-Okoshi, 2000), thus, if food provisioning services are affected, impacts are assumed to be minor. Additionally, the destruction of sedimentary rock and abrasion platforms by the boring activity of *B. proboscidea* (see Qu 5.1) could have implications for coastal erosion processes in the affected areas.

Impacts on cultivated mollusc populations are addressed in the Economic Impacts section.

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	minor	**CONFIDENCE**	low

Comment: Although no information was found on the issue, at the current reported densities, it is possible that the organism may have caused some impact on regulating ecosystem services (coastal erosion rates) through structural effects on abrasion platforms on Helgoland, Germany. Impacts on food provisioning ecosystem services (i.e. shellfish biomass) through mollusk infestations are not known.

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	moderate	**CONFIDENCE**	low

Comment: Although no information has been found on the issue, it can be hypothesized that *B. proboscidea* may impact food provisioning services by reducing shellfish biomass harvested from wild populations for direct consumption or use in aquaculture (mussel and oyster seed, to a lesser extent native abalone, juveniles and adults). Coastal erosion processes may also be intensified (Qu 5.6) in high population density areas. The recreational and aesthetical value of rare, rocky intertidal habitats (e.g. see chalk cliffs) may also be impacted, although there are currently no studies quantifying such services or documenting such impacts (Fletcher et al., 2012). In a worst-case scenario, moderate ecosystem services impacts may be envisaged.

See also Economic impacts below

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	moderate	**CONFIDENCE**	low

Comment:
As regards the “low” score for confidence, *B. proboscidea* has been associated with shellfish, both cultivated and in the wild, in various locations around the world. In the literature, *B. proboscidea* in association with molluscs is primarily described as an interstitial species inhabiting burrows in the spaces between bivalves (e.g. in Argentina, Jaubet *et al*., 2011) but has also been observed as a secondary borer, inhabiting burrows and blisters of other spionid pests, such as *Polydora hoplura* and *Dipolydora capensis* on abalone (works of Simon and colleagues in South Africa; Read, 2004 in New Zealand). It has also been described by Bailey-Brock (2000) as “forming shallow burrows under the lamina of oyster [*Ostrea edulis*] shell valves” but not penetrating the shell all the way to the interior side of the valve. A similar description was offered by Sato-Okoshi (2000) for *B. proboscidea* infesting wild *Crassostrea gigas* from Japan. On the other hand, the species’ ability to bore into soft rock is well documented (Radashevsky *et al*., 2013); additionally, some spionids behave differently across various regions, boring in shells in one region and not boring, but tube-building, in another, with *B. proboscidea* suspected to be one of them (Radashevsky & Pankova, 2013). Since the evidence for shell boring behavior of the species is scant (Radashevsky *et al*., 2019 & personal communication), for the purposes of this RA, it is assumed that *B. proboscidea* does not currently display this trait on a wide scale, the possibility however remains that this may change in the future.

In South Africa, *B. proboscidea* is one of the most problematic spionid pests for abalone *Haliotis midae* aquaculture, infesting most abalone farms, mostly concentrated along the west coast of the country at a mean prevalence of ≈ 61% infested abalone per farm, reaching 100% in some of the farms (Boonzaaier *et al*., 2014). Intensity of infestation per shell was up to ≈ 90 worms/shell (Simon & Booth, 2007). The species may form burrows on the surface of the shell, in crevices on the shell surface or it may occur in the burrows and blisters of other spionid boring pests. In extreme cases it forms ‘mudpacks’ which are covered with a thin layer of nacreous shell in the region of the respiratory pores. These packs usually contain several worms of different sizes and often cause the shell to break along the respiratory pores.

In New Zealand, it was recorded from living shells of commercial shellfish (abalone *Haliotis iris*), in shell blisters together with *P. hoplura*, debri packed, or in shell crevices (Read, 2004).

In Australia, it was found infesting cultured abalone species *Haliotis rubra* & *Haliotis laevigata* but was uncommon (Lleonart, 2002).

Monetary values or estimates for the associated damages/losses were not found, however in 2013 South Africa produced 1470 tonnes of *H. midae*, valued at US$36.31 million (Britz & Venter, 2016), with production projected to increase to 3000 tonnes by 2019 (Britz & Raemaekers, 2015).

Reports of economical losses/damages to cultivated oysters or wild oyster populations due to *B. proboscidea* were not found.

Monetary values for possible management measures of *B. proboscidea* (i.e. management of mudworm infestations on cultivated mollusks) could not be found.

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found". Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minor	**CONFIDENCE**	low

Comments: Economic impacts in the RA area are anticipated to occur primarily in association with cultivated and/or harvested from the wild populations of oysters *Crassostrea gigas* and *Ostrea edulis*, mussels *Mytilus edulis* and *Mytilus galloprovincialis* and to a lesser extent with the abalone species *Haliotis tuberculata*, which supports both a wild fishery in France and small-scale aquaculture in France and Ireland (Robert *et al*., 2013). Aquaculture production of European abalone *H. tuberculata* is based on hatchery produced spat, however transport of juvenile abalone for on-growing takes place between EU countries (Hannon *et al*., 2013).

Even though *B. proboscidea* has already been recorded from intertidal mussel and oyster beds (e.g. Martinez *et al*., 2006; Wijnhoven *et al*., 2017) and from a cultured oyster shell (Radashevsky *et al*., 2019) in the RA area, no economic impacts are reported to date or are expected to have occurred due to low densities. However, it is important to acknowledge that difficulties in the identification of *Boccardia* species have repeatedly led to misidentifications (e.g. Kerckhof & Faasse, 2014; Radashevsky *et al*., 2019) or delayed identification of spionid worms in the case of commercial shellfish infestations (e.g. Simon *et al*., 2006; Simon & Booth, 2007), such that the species may have been overlooked when evaluating impacts of spionid infestations on cultivated oysters. Even if it does not actively burrow into mollusk shells itself, *B. proboscidea* as a secondary occupant of burrows and blisters of other spionid pests can exacerbate the negative impacts of these infestations which include reduced commercial value, growth rate, meat yield and heavy mortality (Royer *et al*., 2006; Sato-Okoshi *et al*., 2012 and references therein).

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	moderate	**CONFIDENCE**	low

Comments:

With respect to shellfish aquaculture, if *B. proboscidea* infests only the surface of the shells, it will not directly affect the biological performance of cultured shellfish, its mere presence however may have negative impacts on the half-shell oyster industry, reducing the presentation/desirability of oysters and their commercial value (Royer *et al*., 2006). If, on the other hand, the species acts as a secondary borer or assumes self-excavating boring behaviour, potential impacts on shellfish aquaculture can be much more severe (see Qu. 5.10 above), but there is high uncertainty associated with such an eventuality.

Potential impacts may extend to wild abalone and mussel seed populations harvested for commercial purposes, as well as oyster spat collectors in the form of dead oyster shell, which constitute settlement habitat for *B. proboscidea* larvae.

Finally, under foreseeable climate change conditions, increased frequency and intensity of storms associated with increased coastal erosion and terrigenous inputs, has the potential to create conditions suitable for spionid outbreaks both on cultured and wild populations of bivalves, i.e. high siltation rates and organic matter inputs that can reduce the fitness of the bivalves and promote both infestations and tube-building and smothering by the worms (Ogburn *et al*., 2007; Clements *et al*., 2017 and references therein; Jaubet *et al*., 2018).

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal	**CONFIDENCE**	medium

Comments: No specific management plans are in place for this particular organism in Europe. For marine invasive species introduced by ballast water/hull fouling and aquaculture, there are considerable management measures at various stages of implementation (see also Management Annex). These costs are not specific for *B. proboscidea* and therefore not included in the score.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	moderate	**CONFIDENCE**	medium

Comments: Considerable costs may be expected if the shellfish aquaculture sector is heavily impacted. A ban of imports or restrictions in the movement of shellfish seed/stock could have potentially significant economic implications for shellfish producers (but the alternative of allowing the risk of introduction may be even more harmful). Mitigation measures to reduce infestation risk and rates, including manipulating planting shore height (e.g. Handley & Bergquist, 1994) and regular cleaning (Nell, 2007; Haupt et al., 2012; Morse et al., 2015) may alter production costs and profits. See also Management Annex. Using hatchery-produced seed may circumvent infestations on seed collectors and reduce the risk of spread of *B. proboscidea* with stock transfers, but comes at a much higher cost (Kamermans, 2008).

General costs related to the prevention of introduction and spread of all marine NIS are not included in the estimation.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minor	**CONFIDENCE**	medium

Comments: Despite the documented impacts of *B. proboscidea* on cultured abalone in South Africa, the abalone aquaculture industry is still thriving (see Qu. 5.9) and no information on consequent social impacts were found. Moreover, abalone infestations by the organism in recent years seem to be under control by the farmers (Carol Simon, personal communication, June 2019), thus any disruption to socio-economic activities is assumed to have been minor at worst. In the RA area, no information on possible social and health impacts was found but no substantial impacts are anticipated to have occurred at the present time due to the low densities of *B. proboscidea* (see Qu. 5.2, 5.10).

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	minor	**CONFIDENCE**	low

Comments: If any social impacts occur in the future in the RA area, these are expected to be associated with the disruption of aquaculture activities and, to a lesser extent, the harvesting of wild mollusks. The information currently at hand indicates that any such impacts are not likely to be stronger than minor (i.e. "Mild short-term reversible effects to identifiable groups, localized").

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	minimal	**CONFIDENCE**	medium

Comments: No such impact information has been found in the literature.

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal	**CONFIDENCE**	medium

Comments: No additional impact information has been found in the literature.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	moderate	**CONFIDENCE**	low

Comments: Information on selective predation, parasitism or pathogens of *B. proboscidea* in the RA area could not be found (but see Qu. 3.5), based however on its invasion history in the RA area and worldwide, the impacts of the species as described in previous sections are not expected to be significantly modified through natural control by other organisms. Besides, its boring behaviour is likely to help the species evade predation.

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	major	**CONFIDENCE**	Low to medium

Comments: At the current reported densities, it is unlikely that the organism will have caused any significant impacts on economic activities (i.e. shellfish culture) in the RA area, although the potential for misidentifications lowers the confidence of this assessment.

Currently, the strongest ecological impacts are reported from Helgoland, Germany, where there are signs of possible displacement of the native polychaete *P. ciliata* by high densities of *B. proboscidea*, as well as concerns about coastal erosion of abrasion platforms by the boring activity of the invader, which has a larger size compared with the native (up to 4.5cm for *B. proboscidea*, up to 3cm for *P. ciliata*).

At organically enriched locations, *B. proboscidea* at moderate densities (e.g. San Sebastian, Spain) may be in competition for space with native mytilids, oysters and barnacles and possibly structuring algae. However, as the species continues spreading in European Seas, particularly the North East Atlantic, the likelihood of population explosions occurring (under current climate conditions) is bound to increase.

Taking into consideration the uncertainties related to predicting population increases of *B. proboscidea*, the species has the potential to cause moderate impacts on biodiversity and ecosystem functioning, particularly in organically enriched areas that favour its proliferation. It can compete for space with native polychaetes, mytilids, oysters and barnacles and possibly structuring algae, and, in a worst-case scenario can smother and displace native species and severely alter native communities. Its boring activity in intertidal firm and hard substrata may have implications for coastal erosion processes, especially in chalk reef habitats, while its tube-building activities can modify soft-sediment habitats. As a pest on wild and cultivated mollusk populations (mussels, oysters and abalone) and depending on the densities achieved, it can affect food provisioning services and cause moderate losses to the aquaculture industry by reducing the desirability and commercial value of oysters (half-shell market), causing serious infestations on native abalone (which however sustains small scale harvesting and culture operations) and possibly interfering with the development of mussel seed beds and seed collectors of mussels and oysters. As an interstitial species on mussel and oyster beds/reefs it has the potential to disrupt the ecological role of these important habitats.

Due to high summer temperatures, strong localized populations sustained by directly developing (adelphophagic) larvae and commonly associated with bivalve transfers, are more likely to develop in the Mediterranean Sea, while ecosystem functioning and structural impacts on a wider scale may be anticipated in the colder, temperate waters of Atlantic Europe, where the species is already established and natural dispersal of planktotrophic larvae by currents will be stronger.

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	moderate	**CONFIDENCE**	Low to medium

Comments: Under foreseeable climate change conditions, increased frequency and intensity of storms associated with increased coastal erosion and terrigenous inputs, has the potential to create conditions suitable for spionid outbreaks both on cultured and wild populations of bivalves, i.e. high siltation rates and organic matter inputs that can reduce the fitness of the bivalves and promote both infestations and tube-building and smothering by the worms, thus exacerbating the likelihood of evidencing more serious environmental and socio-economic impacts, than currently demonstrated.

Additionally, a predicted increase in seawater temperatures under foreseeable climate change conditions is anticipated to lead to a northward expansion of the species, reducing the extent of the areas at risk from localized strong populations and associated impacts in the Mediterranean and the Black Sea and increasing the respective risks in Atlantic Europe.

RISK SUMMARIES

	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction*	likely	high	Occurrence of *B. proboscidea* at locations in the vicinity of ports implies that vessels transfer (either in ballasts or as fouling) is the most plausible pathway of its introduction. Management measures implemented so far (i.e. BWE) have not proven adequate to prevent its introduction in EU marine waters and this will partly continue until the BWMC is fully implemented. On the othe hand aquaculture (contaminant on shellfish imported from outside the RA area) is a very likely mode of its introduction but existing management measures scale down this probability. Conclusively vessels are a likely pathway of *B. proboscidea* introduction, mostly in the North East Atlantic.
Summarise Entry*	likely	medium	as above
Summarise Establishment*	Very likely	high	*Boccardia proboscidea* is already established in the Celtic Seas, the Greater North Sea, the Bay of Biscay and the Iberian Coast and further establishment in these regions is considered very likely. In the Baltic Sea the species will be constrained by low salinities and low winter temperatures, while in the Mediterranean Sea high summer temperatures are likely to favour more localized populations, sustained by directly developing larvae. In the Black Sea, establishment is considered unlikely due to a combination of

			high temperatures and low salinities. Future climate conditions are anticipated to lead to a slight northward expansion of the species, very much limiting the areas suitable for establishment in the Mediterranean and the Black Sea .
Summarise Spread*	rapidly	high	Unaided dispersal (dispersal of larvae with oceanic currents or rafting on natural debris) and multiple pathways of human-aided spread (by vessels, or as pests on shellfish transports) create a considerable potential for rapid spread, in the order of 103 km within 5 years. Planktonic larvae can be transported via ballast waters of commercial vessels and ferryboats but also through bilge waters of leisure and other small craft, while sessile stages (adults brooding eggs and developing larvae) can be widely transferred within fouling communities of ship hulls as well as with bivalve consignments. Natural dispersal of planktotrophic larvae is more pronounced in Atlantic Europe, where further northward spread is expected under future climate conditions; in the Mediterranean Sea bivalve transfers are likely to be the dominant means of spread, both now and in the future.
Summarise Impact*	major	medium	*B. proboscidea* has the potential to cause major impacts on biodiversity and ecosystem functioning, particularly in organically enriched areas that favour its proliferation. It can compete for space with native mytilids, oysters and barnacles and possibly structuring algae, and, in a worst-case scenario can smother and displace native species and alternative communities, as evidenced in other parts of the invaded range. Early reports of species displacement and structural

			impacts on invaded habitats are currently available from Germany and the United Kingdom and potential impacts can be even more serious and irreversible, especially on intertidal soft rock habitats that support unique faunal communities. As a pest on wild and cultivated mollusc populations (mussels, oysters and abalone) it can affect food provisioning services and cause moderate losses to the aquaculture industry.
Conclusion of the risk assessment (overall risk)	High	medium	*Boccardia proboscidea* is already established in the Celtic Seas, the Greater North Sea, the Bay of Biscay and the Iberian Coast and further establishment in these regions is considered very likely. Apart from natural dispersal, vessel related vectors (ballast & bilge waters, hull fouling) have and will continue to aid spread, primarily in Atlantic Europe, while in the Mediterranean Sea bivalve transfers pose the strongest risk for spread. Being a well-known shellfish pest, it may endanger wild and cultivated mollusc populations, particularly oysters, mussels and abalone. In the wild, impactful densities are more likely to develop in organically enriched locations, with implications for native polychaete, mytilid and algal species and associated communities and the structural integrity of the invaded habitats. Such impacts are already manifested in Germany but will most likely have a localised character. Soft rock habitats (e.g. abrasion platforms, chalk cliffs) that harbor unique biological communities are at particular risk of irreversible damage by the boring activity of *B. proboscidea*, which may also have implications for coastal erosion processes.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Belgium	YES	YES	YES	YES	-
Bulgaria	-	-	?	?	-
Croatia	-	-	YES	YES	-
Cyprus	-	-	-	-	-
Denmark	-	-	YES	YES	-
Estonia	-	-	-	-	-
Finland	-	-	-	-	-
France	YES	YES	YES	YES	-
Germany	YES	YES	YES	YES	YES
Greece	-	-	YES	YES	-
Ireland	YES	-	YES	YES	-
Italy	-	-	YES	YES	-
Latvia	-	-	-	-	-
Lithuania	-	-	-	-	-
Malta	-	-	-	-	-
Netherlands	YES	YES	YES	YES	-
Poland	-	-	-	-	-
Portugal	-	-	YES	YES	-
Romania	-	-	?	?	-
Slovenia	-	-	YES	YES	-
Spain	YES	YES	YES	YES	-
Sweden	-	-	-	YES (Skagerrak)	-
United Kingdom	YES	YES	YES	YES	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea	-	-	-	-	-
Black Sea	-	-	-	-	-
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast	YES	YES	YES	YES	-
Celtic Sea	YES	-	YES	YES	-
Greater North Sea	YES	YES	YES	YES	YES
Mediterranean Sea					
Adriatic Sea	-		YES	YES (limited)	-
Aegean-Levantine Sea	-		YES	YES (limited)	-
Ionian Sea and the Central Mediterranean Sea	-	-	YES (limited)	-	-
Western Mediterranean Sea	?	-	YES	YES (limited)	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[7]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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[7] Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al*. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)*

	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[8]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*
		Lifecycle maintenance, habitat	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal;

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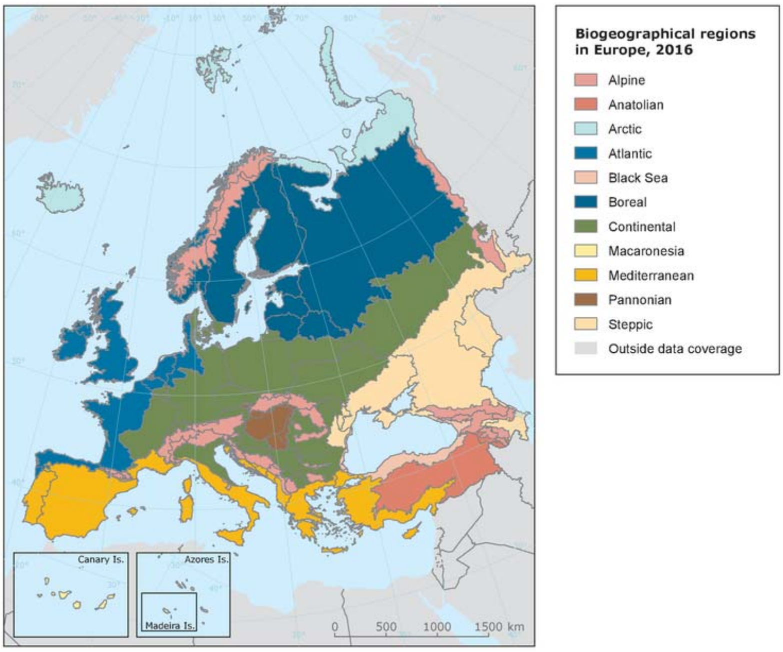
[8] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

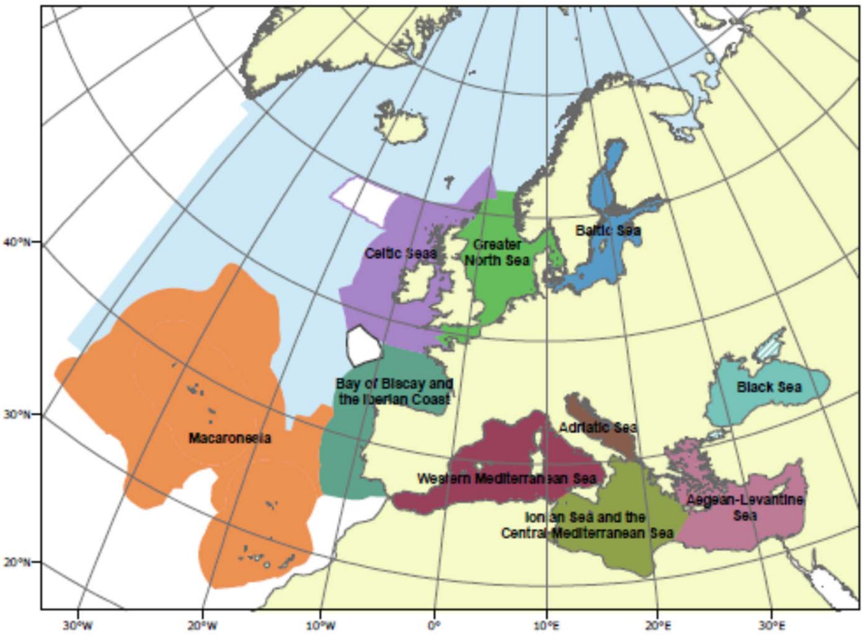
		and gene pool protection	Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*
	Indirect, remote, often indoor interactions with living systems that do not require	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation

	presence in the environmental setting		*Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/
and
https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

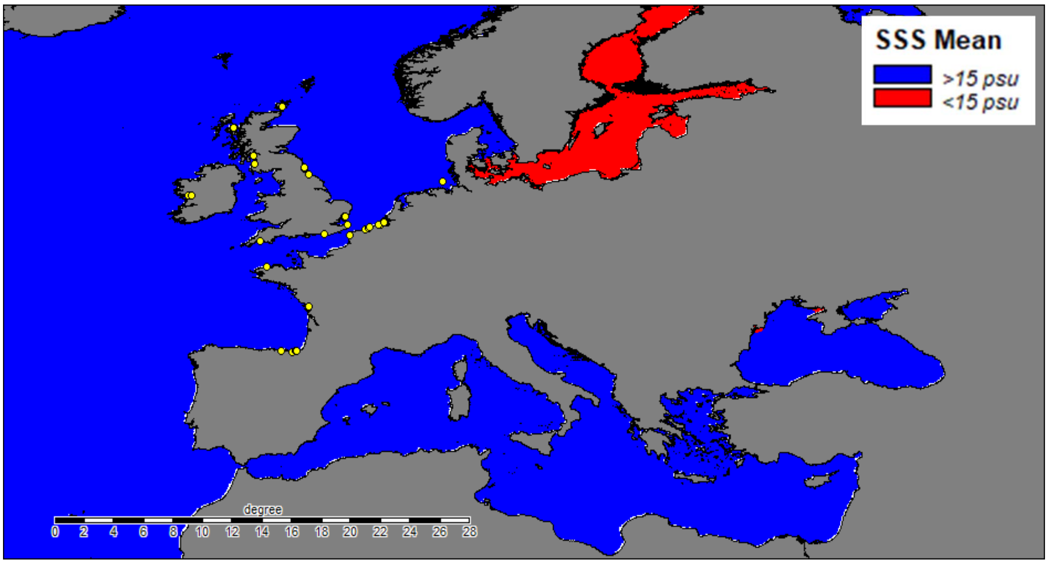
see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

ANNEX VII: *Boccardia proboscidea* physiological requirements & thresholds

Salinity

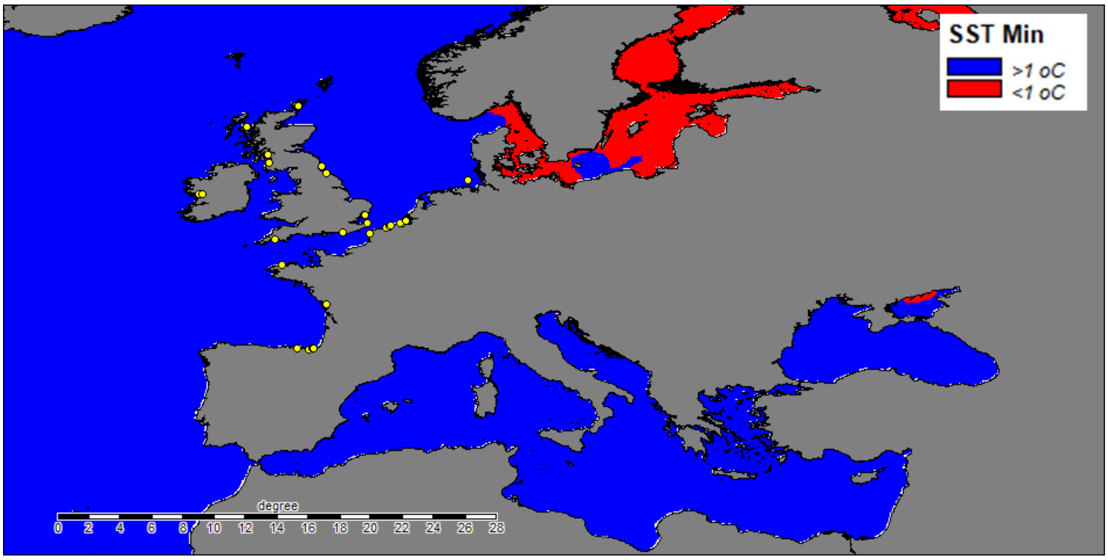
With respect to salinity, in Australia, the species is established in conditions that range from brackish to fully marine (21 to 34.8 psu) (Coleman & Sinclair, 1996) but in laboratory experiments it has been shown to thrive at high salinities of up to 39-40 psu (Hillyard, 1979). Additionally, peak densities in Argentina were observed at salinities between 15-20 psu (Garaffo *et al.*, 2016), associated with increased organic matter conditions at untreated sewage outflows with high freshwater input. Thus, salinity is not expected to pose limitations to survival and establishment at the range of values encountered in the Black Sea (SSS = Sea Surface Salinity of 14-18 psu) but is very likely to become a prohibitive factor in most of the Baltic with the exception of the westernmost parts of the Western Baltic. A salinity threshold of 15 psu was established for mapping and predictive purposes.

Note: All maps in Annex VII were specifically developed for the purposes of this study.



Temperature

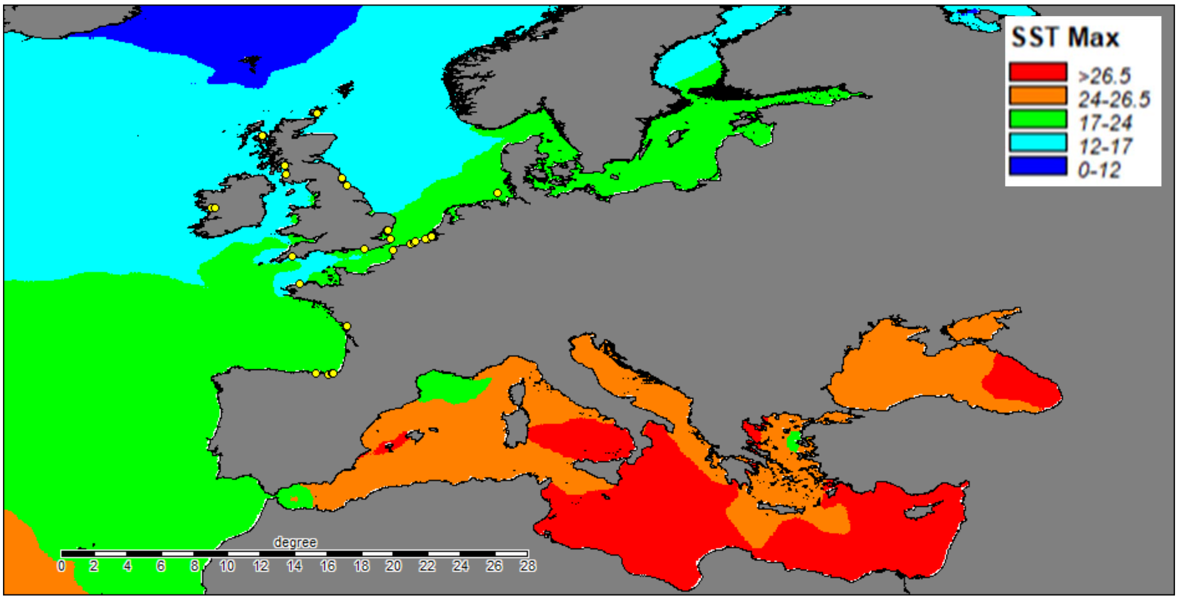
Boccardia proboscidea has been recorded from locations with minimum yearly temperatures as low as 1.15 °C in northern China (Radashevsky *et al*, in press) and 2.6 °C in Japan (Imajima & Hartmann, 1956), it is more regularly encountered however at minimum temperatures between 5-7 °C (Argentina, Canada) and in European waters between 3.7-6 °C. Low winter temperatures in the Baltic (besides the salinity limitations) will hamper establishment in the region.



With regards to high temperature thresholds, the species can be found in Japan, at locations where average temperature of the warmest month reaches ≈26.5 °C (species records from Abe *et al*., 2019 – all temperature values according to BIO-ORACLE data layers, Assis *et al*., 2018, URL: http://www.bio-oracle.org/downloads-to-email.php). Additionally, water temperatures of 24 °C and 28 °C severely reduce the survivorship of planktotrophic and adelphophagic larvae respectively (David and Simon, 2014 - see also Qu 3.9), while at 30 °C embryos do not develop at all (Oyarzun, 2010). The two types of larvae achieve survival optima at different temperatures. At relatively low temperatures (12-17 °C), females release larvae at an earlier stage of development, favouring the survivorship of planktotrophic larvae which manage to escape predation by their adelphophagous siblings (David and Simon, 2014). At higher temperatures, increased developmental rates result in shorter brooding times, increased adelphophagia and higher survivorship of directly developing larvae, potentially leading to strong local populations. For mapping purposes temperature thresholds were determined as follows:

Average temperature of the warmest month

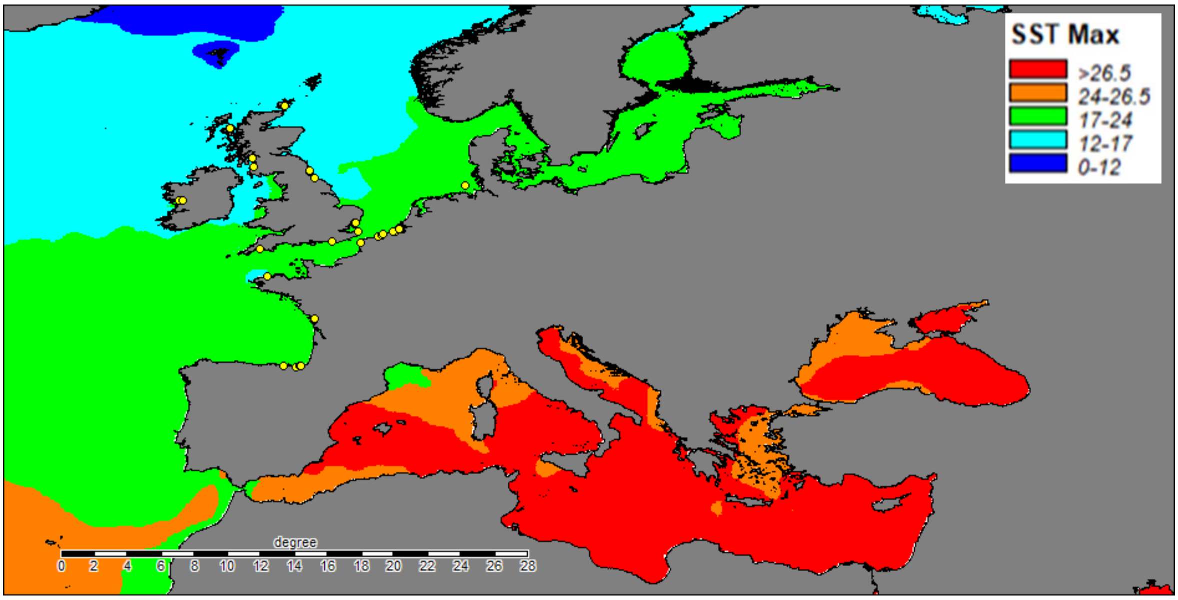
12 °C – the minimum for establishment
12-17 °C – temperatures that favour planktotrophic larvae but do not preclude adelphophages
17-24 °C – temperatures that favour the establishment of both types of larvae
24-26.5 °C – temperatures that favour the establishment of adelphophagic larvae only
> 26.5 °C – prohibitively high for establishment



Accordingly, high summer temperatures in the Levantine and large parts of the Ionian and the Central Mediterranean are expected to limit its distribution in the Mediterranean Sea to the relatively cooler regions. The Mediterranean Sea in general is more susceptible to invasion by adelphophagic larvae.

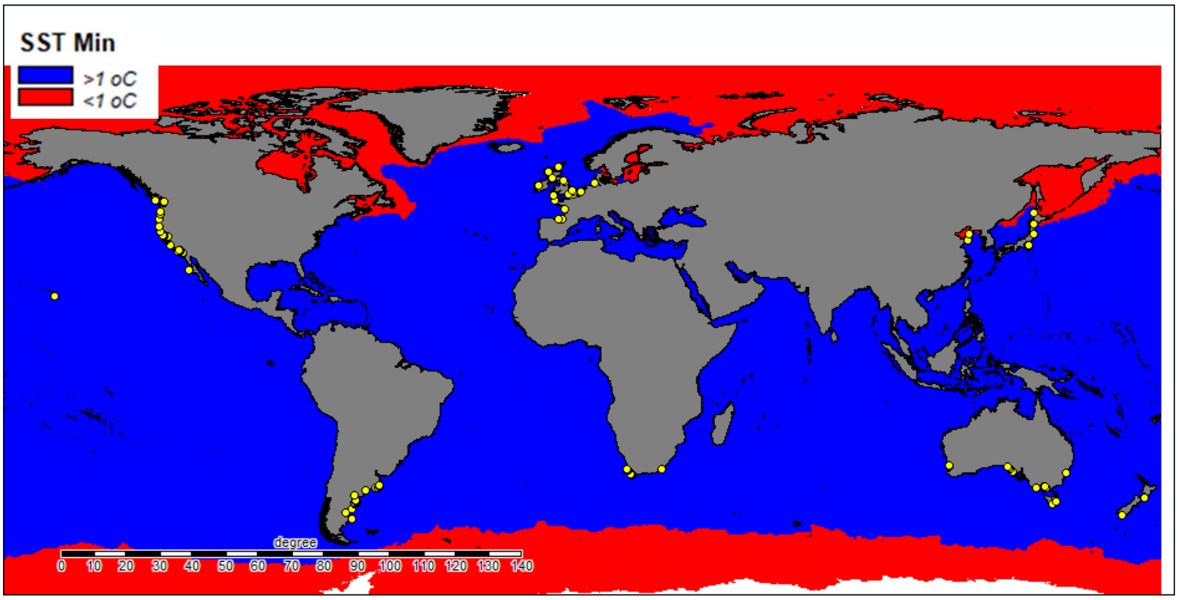
It should be noted that the reproductive period for the species last around 6 months, between March and September in the northern hemisphere (Oyarzun, 2011; Gibson, 1997), thus temperature thresholds based on maximum yearly temperatures may push the predicted distribution slightly northwards and correspond to shorter reproductive periods.

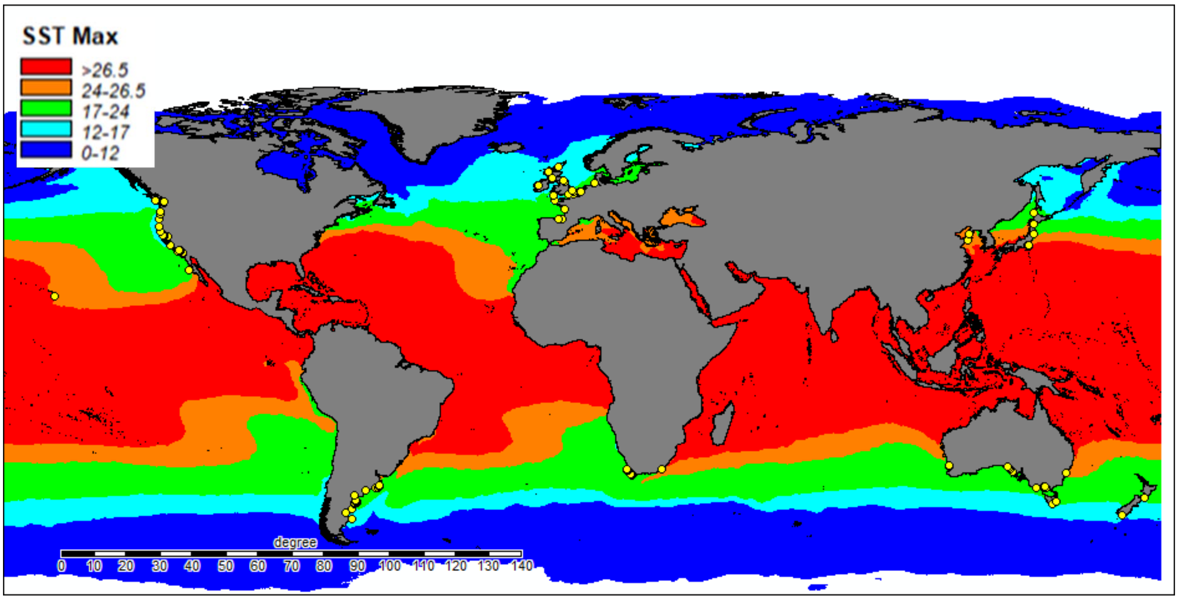
Future scenario (rough estimate based on a maximum increase in seawater temperatures of 0.8 °C by 2065, according to the medium timeframe RCP 4.5 scenario).



Note for the Black Sea: the species distribution model (SDM), presented in Annex VIII, predicts a rather low likelihood of occurrence in the Black Sea, limited primarily by low salinities in the region (see map of limiting factors in Annex VIII). It is believed however that the model has a low sensitivity for salinity as a predictive layer due to the resolution of the underlying data layer, which would not pick up local salinity differences (this is the case for example for Argentina, where peak densities were observed at salinities between 15-20 psu (Garaffo *et al*., 2016), associated with increased organic matter conditions at untreated sewage outflows with high freshwater input. The global salinity layer however indicates salinities in the range of 32psu at the same locations.) Maximum summer temperatures in the western Black Sea are very close to the absolute limit we set for establishment and salinities are within the acceptable range for *B. proboscidea* (assumed here as >15psu) in most of the Black Sea and especially the western part (salinity range 14-19psu). In any case, the species will most likely find itself at the edge of its physiological tolerance both in terms of temperature and salinity simultaneously, facing challenging conditions.

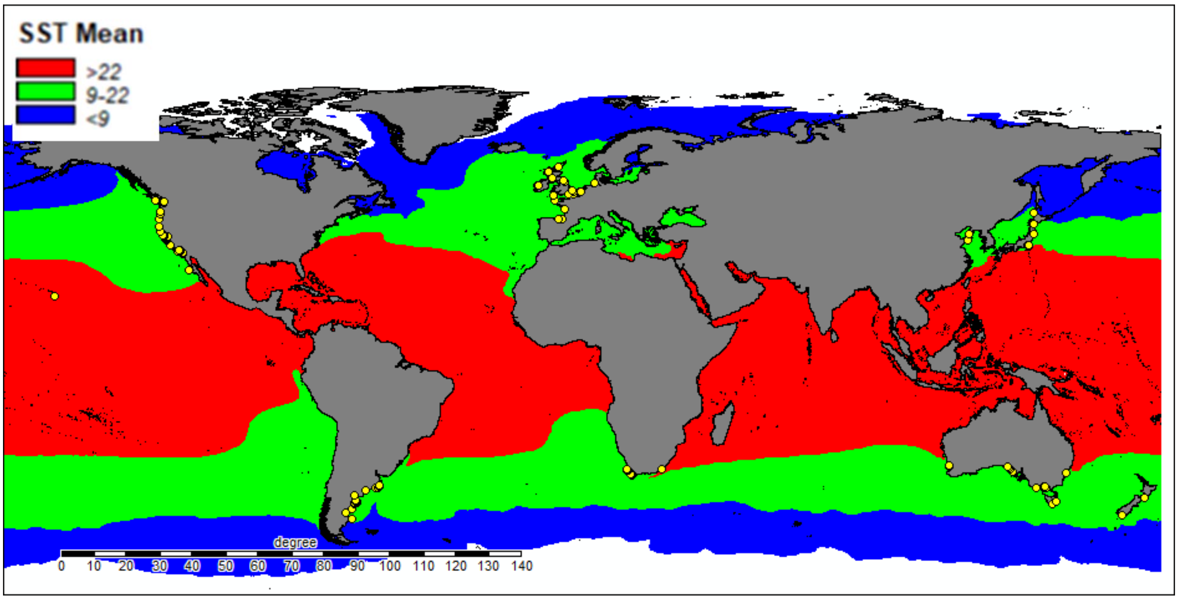
Global Maps Current



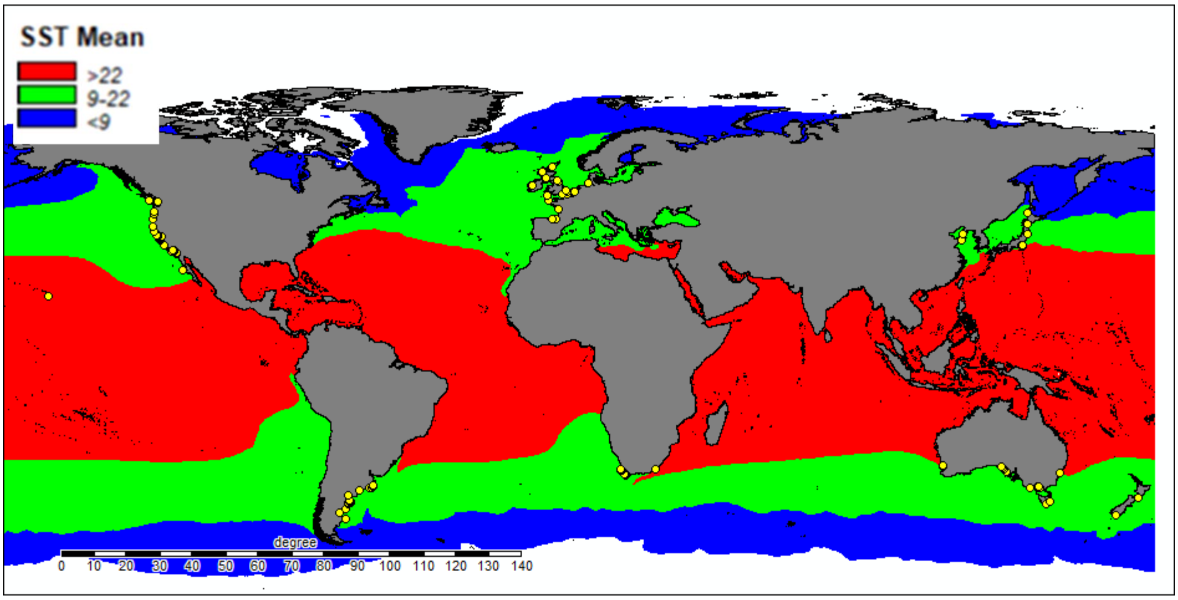


As an alternative to using min and max yearly temperatures to estimate the potential distribution of the species, maps are also presented with average yearly temperature (SST Mean) as a "predictor". Thresholds correspond to the minimum (9 °C) and maximum (22 °C) mean yearly SST values encountered by *B. proboscidea*, based on global occurrence records and BIO-ORACLE2 data layers as above. Green areas represent climatic conditions suitable for establishment.

All the produced maps are only indicative of potential distributions and were used to inform and supplement a Species Distribution Model, presented in Annex VIII.



Future SST Mean (+ 0.8 °C)



ANNEX VIII: Projection of climatic suitability for *Boccardia proboscidea* establishment

Björn Beckmann, Marika Galanidi, Argyro Zenetos, Vasily Radashevsky, Beth Purse and Dan Chapman

31 October 2019

Aim

To project the suitability for potential establishment of *Boccardia proboscidea* in Europe, under current and predicted future climatic conditions. The model and all its outputs (Tables and Figures of Annex VIII) were specifically developed during the framework of this study.

Data for modelling

Species occurrence data were provided by the risk assessment team. The records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 104 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Polychaeta records held by GBIF was also compiled on the same grid (Figure 1b).

Predictors describing the marine environment were selected from the 'Bio-ORACLE' set of GIS rasters providing geophysical, biotic and environmental data for surface and benthic marine realms (Tyberghein et al., 2012, Assis et al. 2017), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Boccardia proboscidea*, the following climate variables were used in the modelling, with all except diffuse attenuation measured at the sea surface:

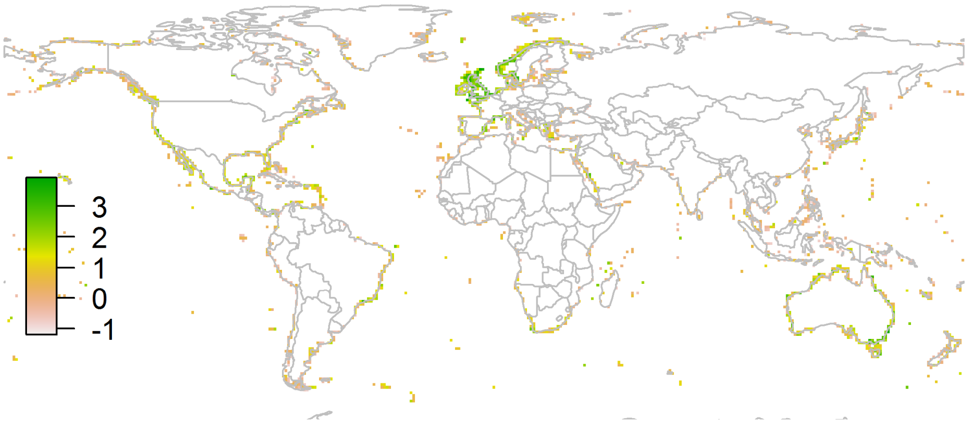
- Maximum long-term temperature (templtmax_ss)
- Mean salinity (salinitymean_ss)
- Mean current velocity (curvelmean_ss)
- Maximum primary production (ppmax_ss)
- Mean diffuse attenuation (damean)

To estimate the effect of climate change on the potential distribution of *Boccardia proboscidea*, equivalent modelled future conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. Projections for the 2070s were calculated as averages of projections for the 2040s and 2090s (which are the time periods available on Bio-ORACLE). Future projections are not available for dissolved oxygen concentration, primary production and diffuse attenuation - for these, current values were used.

Figure 1. (a) Occurrence records obtained for *Boccardia proboscidea* and used in the modelling, showing native and invaded distributions. (b) The recording density of Polychaeta on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling

(b) Estimated recording effort (log10-scaled)



Species distribution model

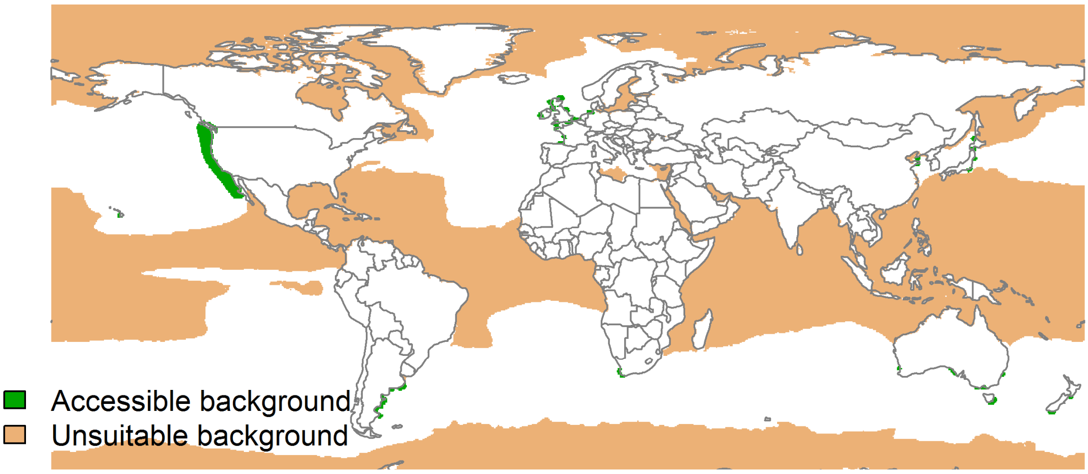
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1 (Thuiller et al., 2019, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore, the background sampling region included:

- The area accessible by native *Boccardia proboscidea* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 300km buffer around the native range occurrences; AND
- A 100km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Boccardia proboscidea* at the spatial scale of the model:
  - Minimum long-term temperature (templtmin_ss) < 1.5
  - Maximum long-term temperature (templtmax_ss) > 27.5
  - Mean salinity (salinitymean_ss) < 15

Altogether, 0% of occurrence grid cells were located in the unsuitable background region.

Within the background region, 10 samples of 1000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Boccardia proboscidea*. Samples were taken from a 300km buffer around the native range and a 100km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the background sample was much larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams &

Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).

- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCdist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Boccardia proboscidea* was most strongly determined by Maximum long-term temperature (templtmax_ss), accounting for 72.9% of variation explained, followed by Mean salinity (salinitymean_ss) (14.2%), Mean current velocity (curvelmean_ss) (5.6%), Maximum primary production (ppmax_ss) (5.6%) and Mean diffuse attenuation (damean) (1.7%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (ROC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

					variable importance (%)				
Algorithm	AUC	Kappa	TSS	Used in the ensemble	Maximum long-term temperature (templtmax_ss)	Mean salinity (salinitymean_ss)	Mean current velocity (curvelmean_ss)	Maximum primary production (ppmax_ss)	Mean diffuse attenuation (damean)
GLM	0.953	0.615	0.844	yes	86.0	4.2	6.9	2.0	1.0
GAM	0.947	0.638	0.852	no	76.9	8.2	7.3	6.4	1.2
ANN	0.952	0.655	0.867	yes	72.0	15.7	5.8	4.5	2.1
GBM	0.963	0.678	0.854	yes	76.3	12.4	6.5	3.6	1.1
MARS	0.957	0.681	0.854	yes	73.2	16.5	5.3	5.0	0.0
RF	0.956	0.697	0.831	yes	57.0	22.5	3.3	12.9	4.3
Maxent	0.921	0.630	0.765	no	59.2	12.6	7.8	15.3	5.1
Ensemble	**0.969**	**0.693**	**0.869**		**72.9**	**14.2**	**5.6**	**5.6**	**1.7**

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

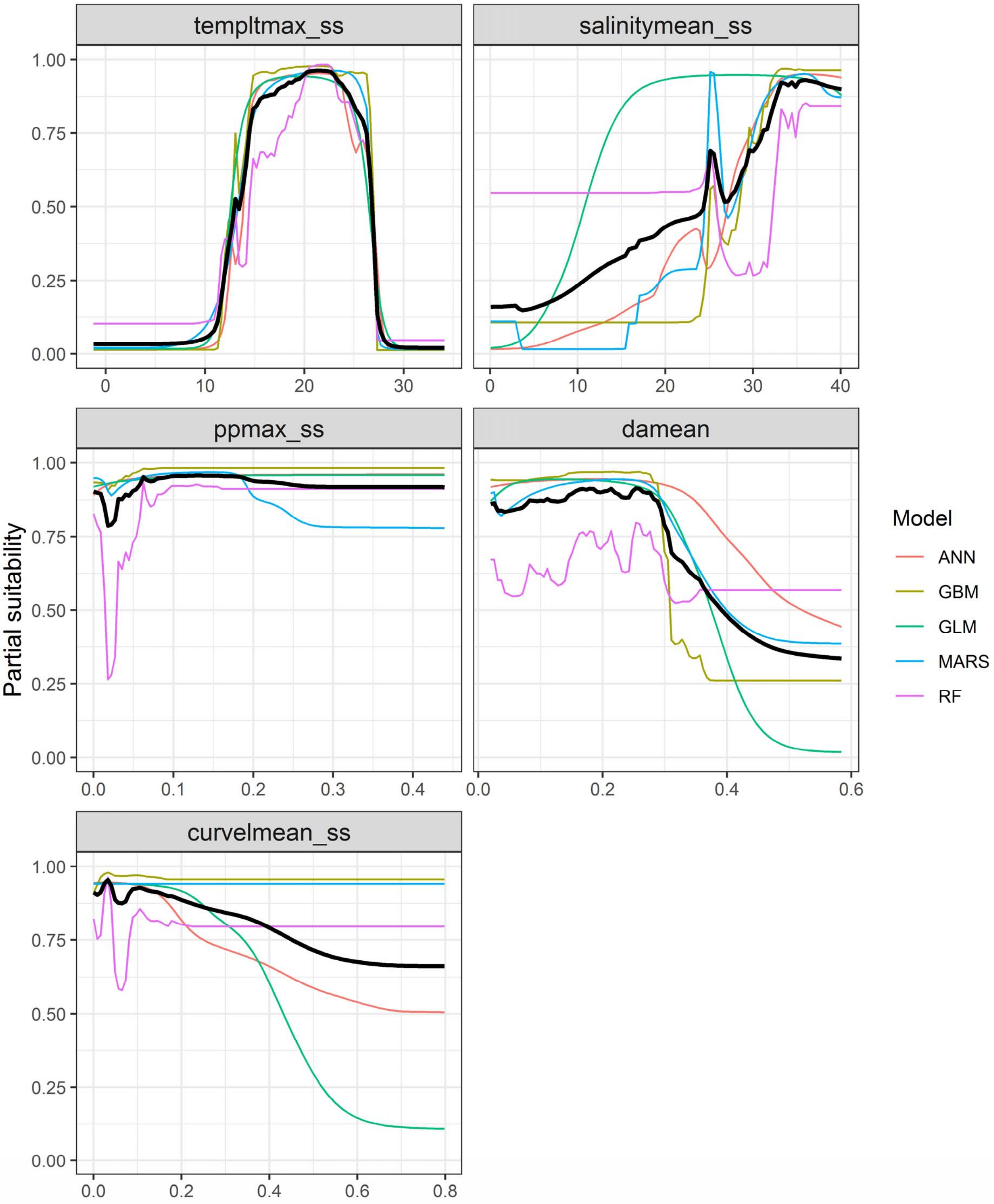
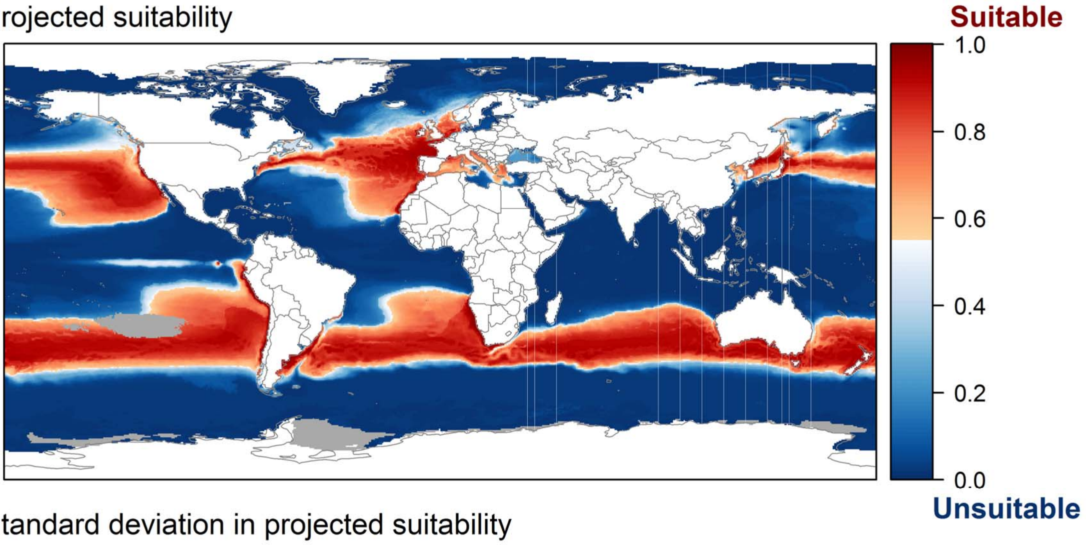


Figure 4. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.55 may be suitable for the species. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

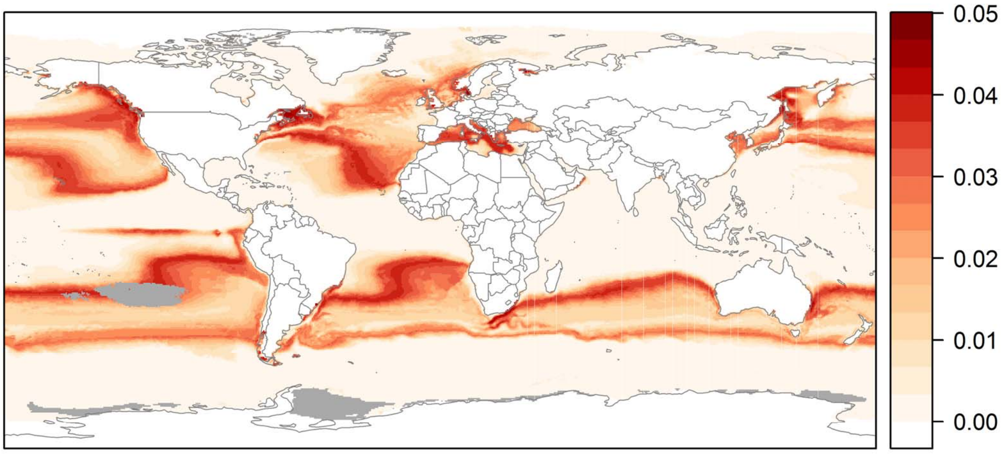
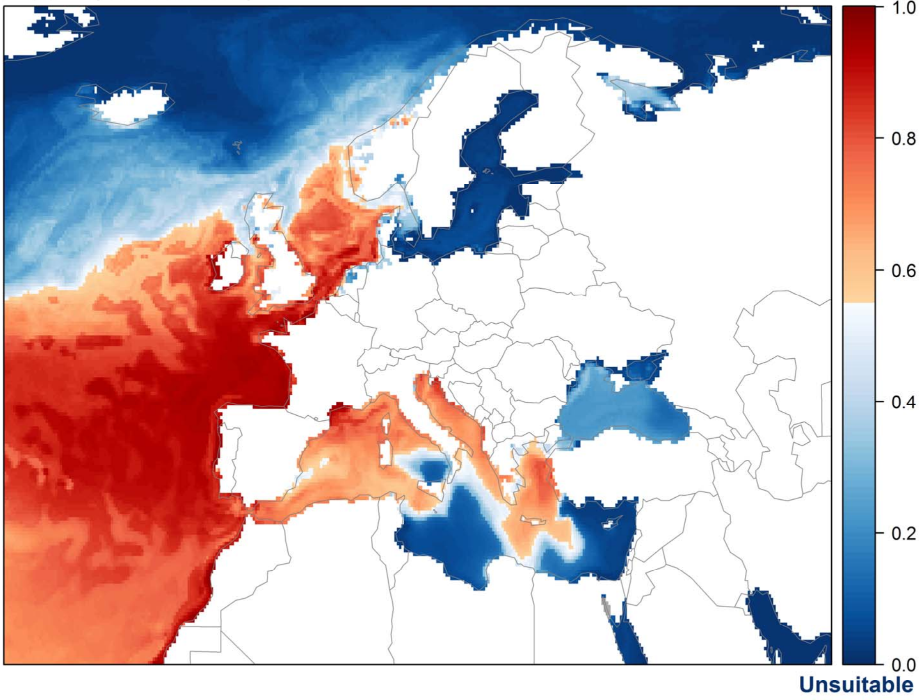
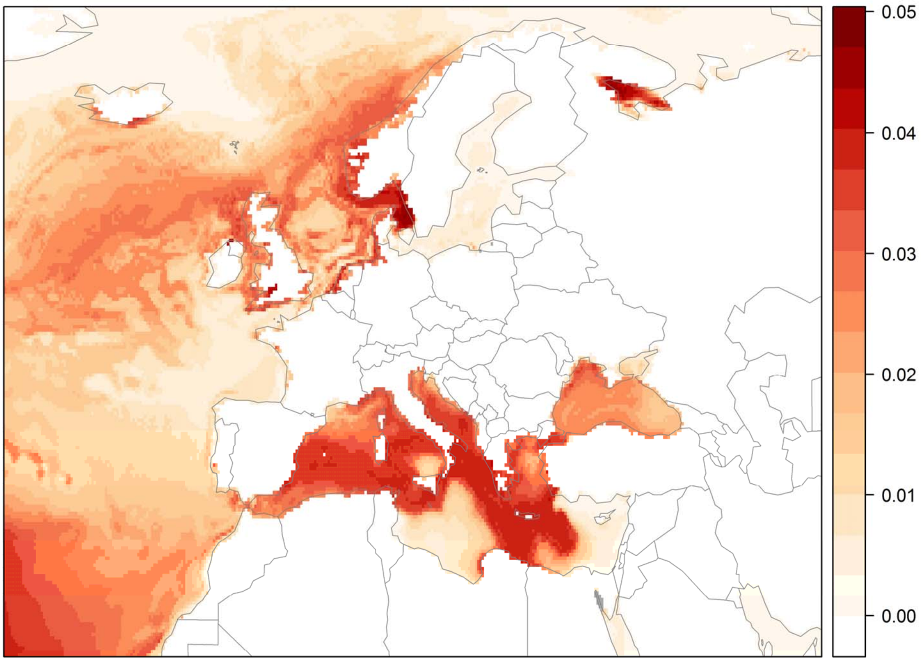


Figure 5. (a) Projected current suitability for *Boccardia proboscidea* establishment in Europe and the Mediterranean region. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



(a) Projected suitability



(b) Standard deviation in projected suitability

Figure 6. The most strongly limiting factors for *Boccardia proboscidea* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

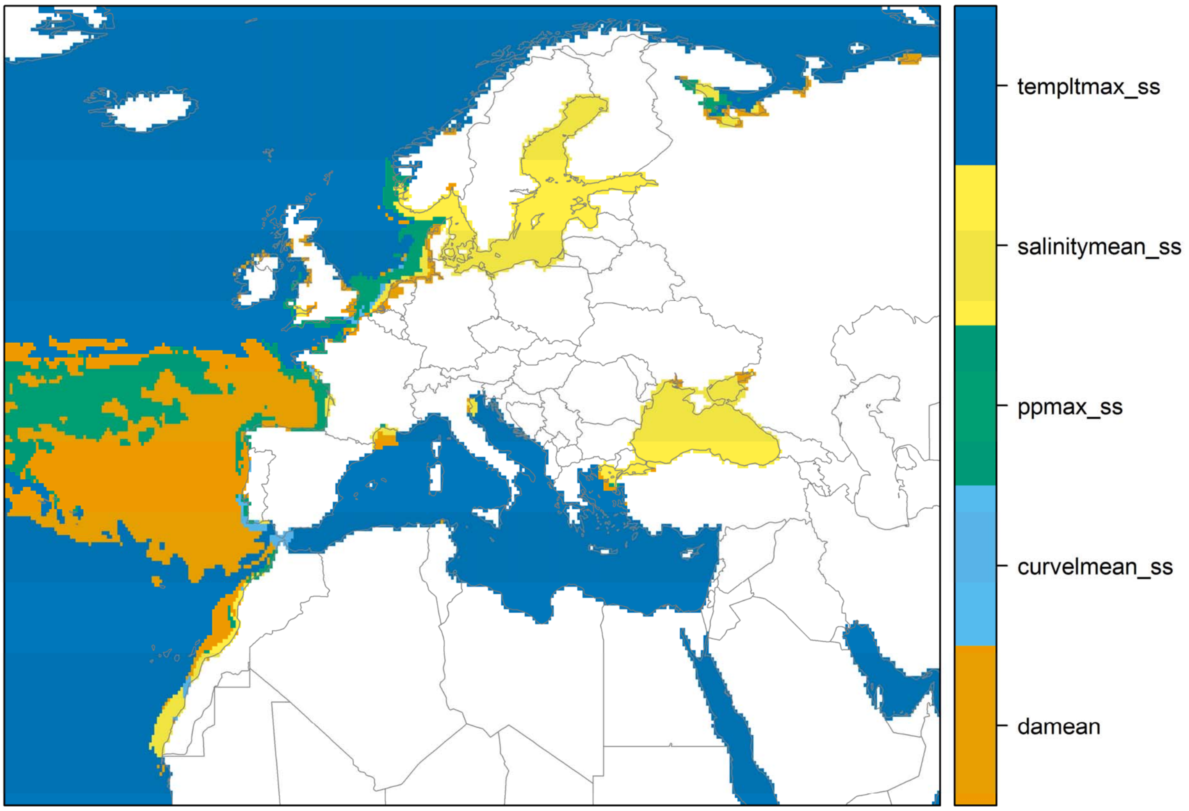
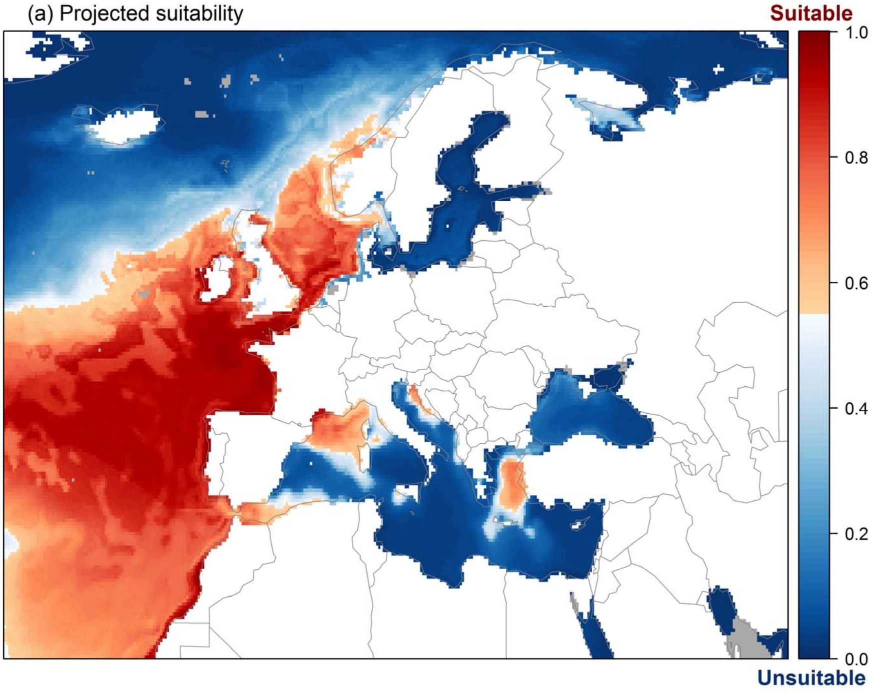


Figure 7. (a) Projected suitability for *Boccardia proboscidea* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

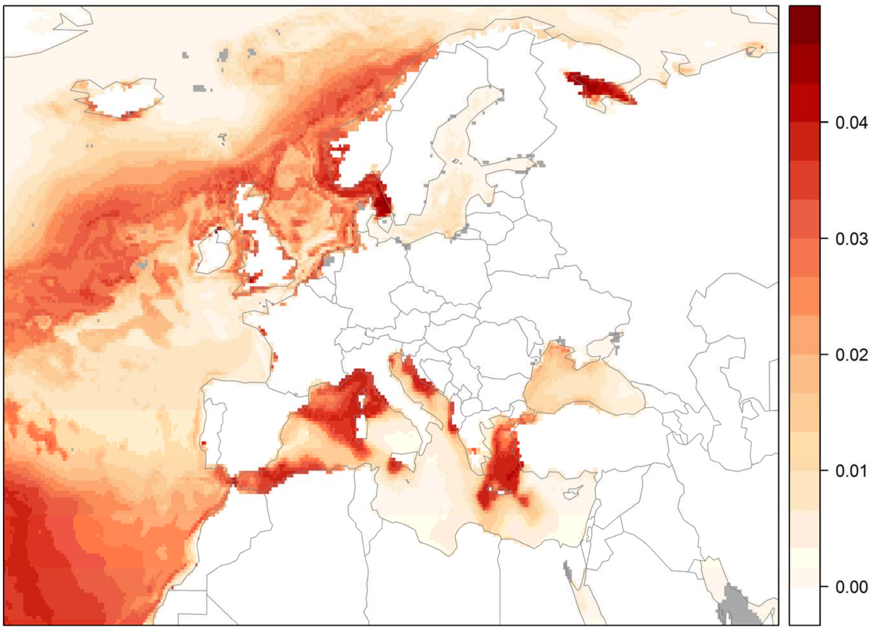
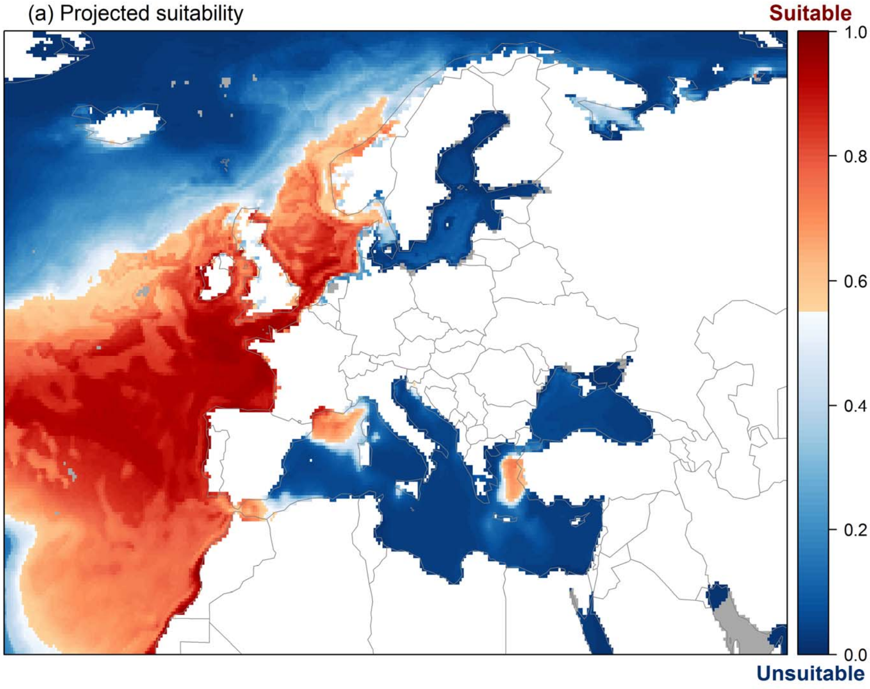


Figure 8. (a) Projected suitability for *Boccardia proboscidea* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

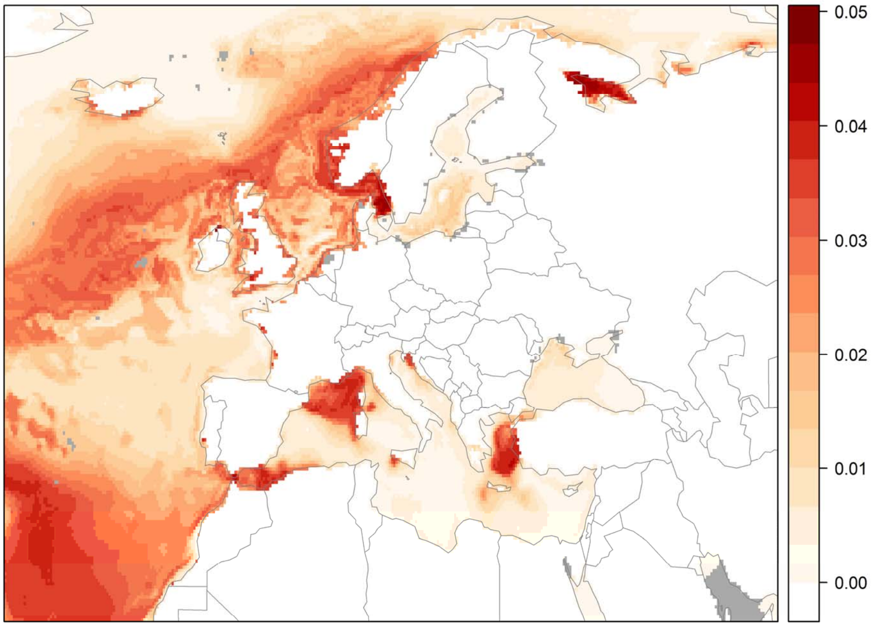


Figure 9. Variation in projected suitability for *Boccardia proboscidea* establishment among marine subregions of Europe (https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf/at_download/file). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown.

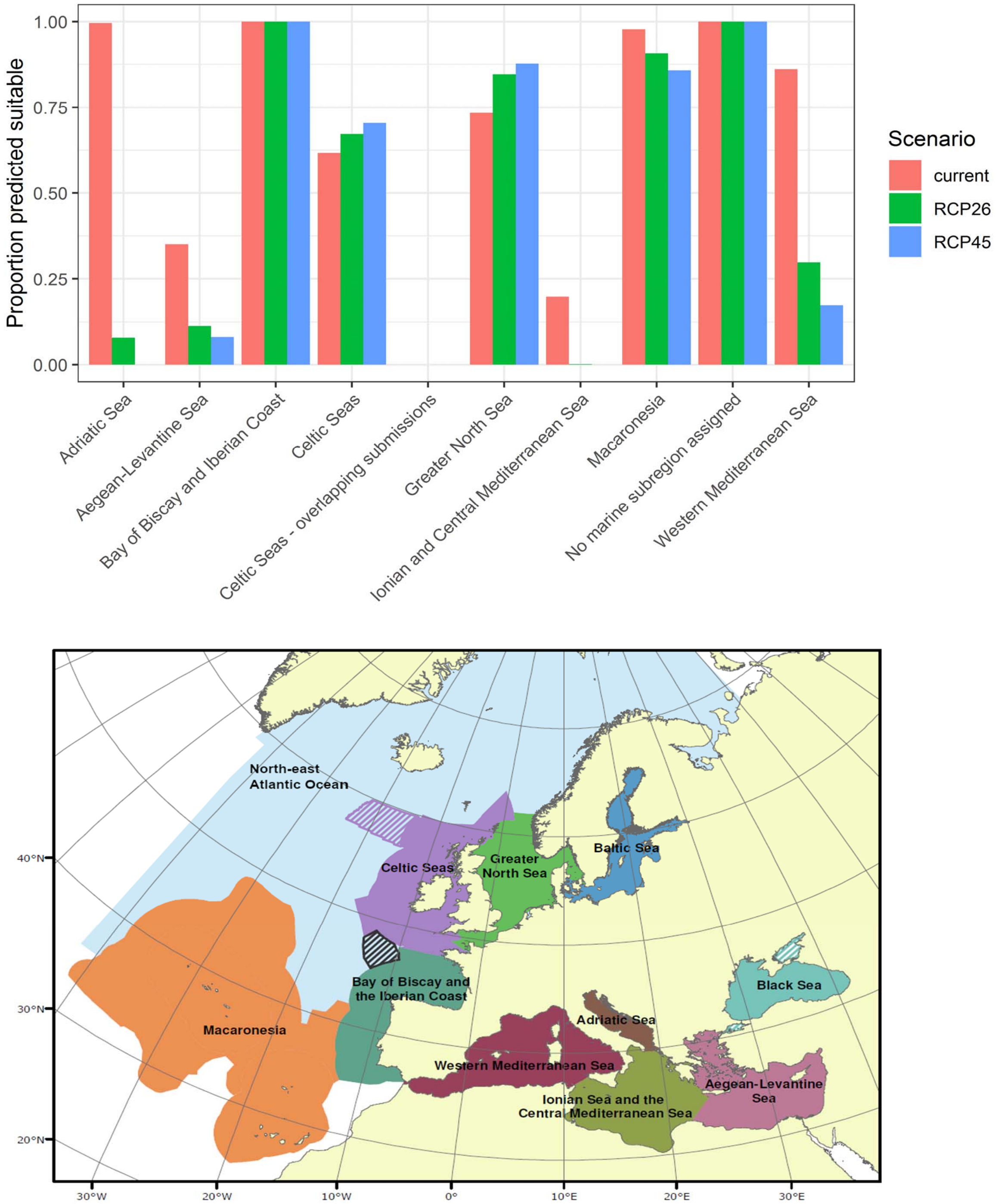
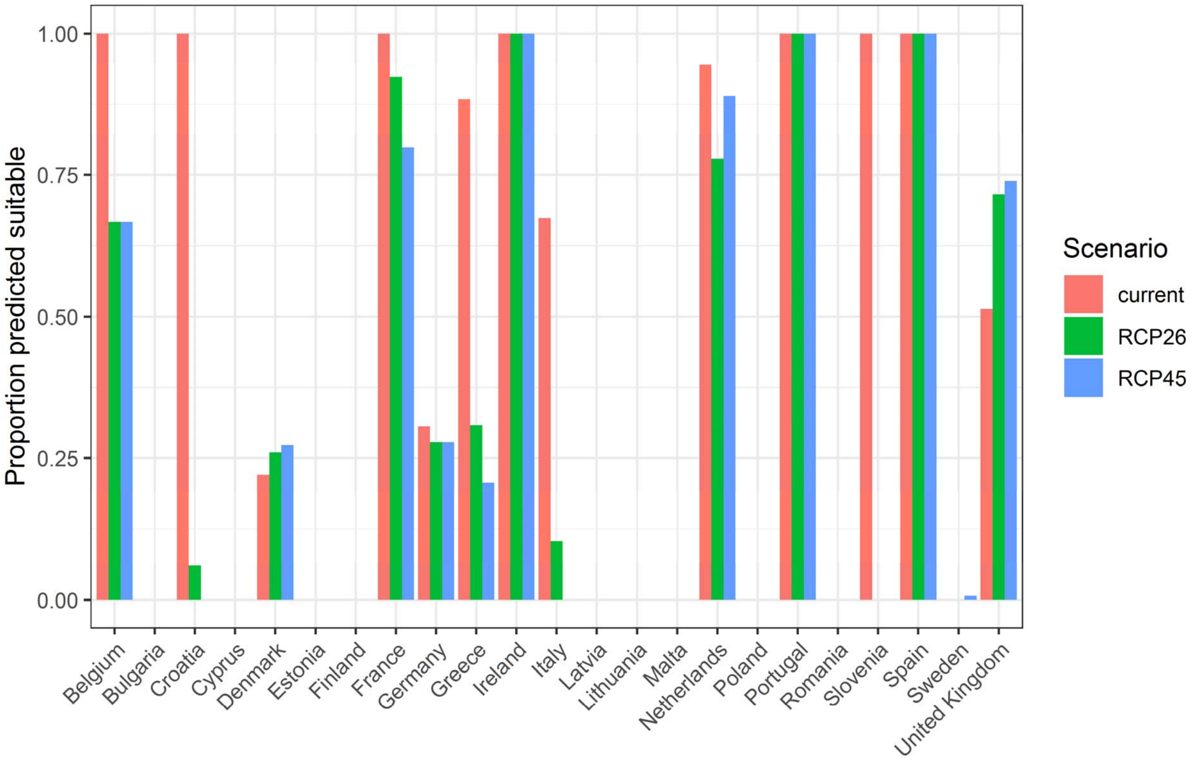


Figure 10. Variation in projected suitability for *Boccardia proboscidea* establishment among the 12-nautical-mile national waters of European Union countries. The bar plots show the proportion of grid cells in each country's waters classified as suitable in the current climate and projected climates for the 2070s under two RCP emissions scenarios.



Caveats to the modelling

To remove spatial recording biases, the selection of the background sample was weighted by the density of Polychaeta records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as sea depth were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Boccardia proboscidea***
Species (common name)	**A burrowing spionid worm**
Author(s)	**Marika Galanidi, Argyro Zenetos**
Date Completed	**31/09/2019**
Reviewer	**Peter Robertson**

Summary [1]

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Boccardia proboscidea is already established in the Risk Assessment area (in Atlantic Europe) and is spreading unaided as well as through mainly ship-mediated pathways (ballast waters, hull fouling, bilge waters) and as an aquaculture pest on molluscs. The risk of further introductions and spread through ballast waters will be much lower once the Ballast Water Management Convention is fully implemented (presumably by 2024), as compliance with the International Maritime Organisation's (IMO) D2 standard can decrease larval concentrations to undetectable levels, significantly reducing propagule (larval) pressure, however the species can keep spreading through other means. Hull fouling is less strictly regulated and is applied on a voluntary basis; dry docking at appropriate intervals can be effective but is costly, while emerging in-water cleaning technologies with capture and treatment of the fouling debris that can provide more cost-effective alternatives are being actively explored in order to avoid the risk of releasing propagules in the environment. A stricter regulatory framework needs to be implemented if biofouling of both commercial and recreational vessels is to be effectively managed. Bilge waters of recreational and other small vessels are not managed for invasive alien species (IAS) but this pathway could be addressed with awareness raising campaigns to boat owners and the promotion/adoption of relatively simple measures, such as discharge of bilge water in the source region or retention

and subsequent treatment. Current levels of compliance with other, voluntary biosecurity measures (for hull fouling) by leisure craft owners however, indicate that voluntary guidelines are not sufficiently adopted to eliminate the risk of IAS spread. Shellfish imports from countries outside the EU are strictly managed and uncommon in the past 1-2 decades, this pathway however is still very relevant for spread within the RA, unless national/regional regulations provide a stricter regulatory framework than Council Regulation (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture, especially with respect to oyster *Crassostrea gigas* (*Magallana* gigas) transfers. Restrictions on transfers based on the risk associated with the source areas is an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk IAS are available. For *B. proboscidea* in particular, obtaining such data had proven problematic in the past, as the species is easily misidentified. A recent surge of interest has resulted in a large number of backdated, previously unreported and new records; further early detection systems are better designed with a focus on the training of professionals who are likely to encounter/collect it during the course of monitoring or other marine survey activities, as well as potentially affected stakeholders (i.e. shellfish growers). Due to difficulties in detection and identification but also due to *B. proboscidea*'s capabilities for both long-range dispersal and almost continuous local recruitment throughout the year, the eradication of the species in the wild is considered unlikely. Besides monitoring of typical NIS[a] hotspots (e.g. ports, marinas, aquaculture plots), monitoring for *B. proboscidea* should include organically enriched habitats (in the vicinity of introduction hotspots), such as for example sewage outflows, that typically foster dense populations. Considering that, for *B. proboscidea*, the most critical factor for the manifestation of impacts on biodiversity and ecosystem services is the development of dense populations, maintaining nutrient and organic matter discharges at levels that do not favour outbreaks of opportunistic polychaetes may be an effective management measure in the wild, particularly at source point locations. In shellfish culture systems, a large suite of measures is available to the industry to combat mudworm infestations; the most effective ones and less harmful for the cultured organisms as well as the recipient ecosystem involve methods such as planting shore height manipulations, jet-blasting with sea water, immersion in fresh water or heated seawater, air drying of stocks, etc., depending on the cultivated species. Chemical treatments in aquaculture systems are not advised but may be warranted for the acute suppression of locally abundant populations if nearby sensitive areas (e.g. of conservation concern) are threatened.

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)[3]**	**Level of confidence[4]**

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[a] NIS: non-indigenous species, term used in the Marine Strategy Framework Directive, synonym of "alien species" as used in the framework of Regulation (EU) 1143/2014

Methods to achieve prevention[5]	**P1. Managing the pathway "Ships' Ballast Water"** Regulation (EU) No 1143/2014 on the prevention and management of the introduction and spread of invasive alien species recognises the IMO Ballast Water Management Convention as one of the possible management measures for invasive species of concern. This entered into force in September 2017 and requires ships in international traffic to apply ballast water management measures, such as ballast water exchange (D-1 standard for an interim period) and fulfil a certain discharge standard (D-2 standard according to the ship specific application schedule). The D1 standard requires ships to exchange a minimum of 95% ballast water volume whenever possible at least 200 nautical miles (nm) from the nearest land and in water depths of at least 200 metres. When this is not possible, the BWE shall be conducted at least 50 nm from the nearest land and in waters at	The D1 standard on Ballast Water Exchange (BWE) is currently practiced and can reduce the risk of introduction and spread for a wide range of species, potentially transferred in ballast water, besides *B. proboscidea*. BWE can reduce the concentration of alien organisms in ballast waters discharged in coastal waters by 80–95% (Ruiz & Reid, 2007). Its application, however, has severe limitations, primarily dependant on geography (David et al., 2007), weather conditions or other safety restrictions (ship instability). In addition, BWE effectiveness is influenced by tank structure and the skill and experience of the ship crew, while it cannot be used to manage ballast water of vessels on shorter coastal (non-transoceanic) voyages (Bailey 2015). Efficiencies of various technologies utilised for ballast water treatment are reviewed in Tsolaki and Diamadopoulos (2009) and can vary with treatment method, but the application of many combined methods (e.g., Filtration+UV or Hydroclone+chemical disinfectant) can decrease live zooplankton to undetectable levels, practically diminishing propagule pressure. Costs related to Ballast Water Management and the implementation of IMO's D2 Standard are not specific to *B. proboscidea* but refer to all marine NIS. The cost of installing Ballast Water Management Systems will be borne by the shipping companies. Installation costs can vary from €140K-€1.675m per ship (figure reported in DEFRA (2012), after consultation with the UK Marine Coastguard Agency). Significant costs can be associated with the ratification of the Ballast Water Management Convention in ensuring its	**Medium** Confidence depends on proper implementation of ballast water treatment and discharge.

	least 200 metres in depth (BWMC Guideline 6). D-2 states that ships shall discharge (in relation to the organism size range of interest for *B. proboscidea*): less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension. This can be achieved through mechanical (filtration, separation), physical (heat treatment, ozone, UV light) and chemical methods (biocides). The BWMC entered into force in September 2017, but full implementation will take some years (as late as 2024 – see http://maritime-executive.com/article/imo-pushes-back-ballast-water-compliance-dates). Eventually, most ships will need to install an on-board ballast water treatment system, and all ships will be required to carry a ballast water record book and an international ballast water management certificate.	compliance, related to, for example, planning, monitoring, enforcement and capacity-building. As an example, in Croatia, approximately €1.26 million will be incurred by the state for institutional capacity building and in fulfilment of its flag State and port State obligations (Interwies & Khuchua, 2017). This is broken down into €470k from flag state obligations (with 150 registered ships in 2015) and €482k from port state obligations (with 6 major ports). The remaining €308k are the preparatory phase costs. Compliance costs will need to be incurred anyway to enable trade with other countries. Additionally, Regulations A4 and A3 of the BMWC enable the granting of exemptions from the requirement for BWM (provisions made in the BWMC for regular routes and other cases – Olenin et al., 2016). Such requests must fulfil certain criteria and require a risk assessment (HELCOM, 2014). HELCOM has developed an ONLINE BALLAST WATER RISK ASSESSMENT TOOL to aid in the identification of shipping routes that may fulfil the criteria for exemptions (http://jointbwmexemptions.org/).	

	P2. Managing the pathway “Ships’ Hull Fouling” Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species were adopted on 15 July 2011 [RESOLUTION MEPC.207(62)] - "2011 Guidelines for the Control and Management of Ships' Biofouling to Minimize the Transfer of Invasive Aquatic Species". The guidelines are voluntary and apply to all ships. Commercial ship-owners have a strong interest in having their vessels cleaned in order to decrease their fuel consumption but dry-docking frequency is determined by performance (fuel consumption below a certain threshold) and can range from 0.5-5 years (Bohn et al., 2016). Recreational crafts shorter than 24 m in length may instead find relevant guidance in IMO's 2012 document "Guidance for	Invasion risk is associated with the time interval between dry-docking events and the residence time of the ship in port (Galil et al., 2019). Vessel cleaning during dry-docking in a shipyard generates a very low biosecurity risk because the debris is sent to local deposit and residue water from cleaning is collected (Bohn et al., 2016). Maintenance during dry-docking also involves the re-application of anti-fouling paint, which seems to be efficient for up to 1-1.5 years – thereafter heavy fouling can start occurring (Sylvester et al., 2011; Frey et al., 2014). Tributyltin (TBT), one of the most potent/effective anti-foulants, had devastating effects on marine life, causing, among other impacts, imposex in dogwhelks *Nucella lapillus*, mass mortalities in oyster larvae and severe malformations in oyster shells (Santillo et al., 2001), was finally banned in 2008 when the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, 2001 came into force. With less effective anti-fouling systems in place, ships’ fouling continues to pose serious invasion risks (Frey et al., 2014). Vessels with larger docking intervals (up to 5 years) increasingly choose intermediate cleaning of the hull with in-water technologies (Bohn et al., 2016). In-water cleaning of hulls, especially without capturing the biofouling debris, might represent a higher risk of introducing NIS relative to land-based cleaning in dry-docks with land based waste disposal since physical disturbance of the fouling communities may trigger the release of propagules or viable gametes (Hopkins & Forrest, 2008). Dislodged individuals are	**Medium** Removing biofouling material and ensuring fouling free vessel surfaces reduces propagule pressure and the associated risks of alien species translocations. Nevertheless, there does not appear to be any comprehensive analysis of the compliance levels or overall efficacy of the existing, voluntary biofouling guidelines in reducing alien species introductions & spread (Hayes et al., 2019). Early information from New Zealand indicates a

	Minimizing the Transfer of Invasive Aquatic Species as Biofouling (Hull fouling) for Recreational Craft" (MEPC, 2012). The two main practices for the removal of biofouling from ships' hull are: • Dry docks • In-water cleaning (IWC), used as an additional tool to dry-docking; it can be combined with loading/unloading activities, is faster and can cost as little as 1/5 the cost of dry docking (Hagan et al., 2014). It should be noted that certain regional regulations regarding biofouling management have already entered into force, e.g. in Australia, New Zealand, parts of the USA and the Galapagos Marine Reserve (Zabin *et al.,* 2018).	likely to settle on surrounding benthos and if surrounding habitat is suitable, may spread and become established. Recent advances in in-water cleaning technologies overcome this problem with systems that capture and/or render the debris non-viable via treatment with heat, UV radiation or biocides (Morrisey & Woods, 2015; Zabin et al., 2016), such systems though sometimes fail to contain all of the removed debris and may damage anti-fouling coatings, rendering them less effective (Scianni & Georgiades, 2019 and references therein). On the other hand, in-water cleaning of micro-fouling (i.e. slime layer) with little to no-abrasive methods was found to result in acceptable levels of both biosecurity (i.e. alien species) and chemical contamination risk for biocides in New Zealand (Morrissey et al., 2013; DOE/MPI, 2015). With respect to recreational craft, Gittenberger et al., (2017) concluded that most recommendations in the IMO guidelines will indeed minimize the risk of non-native species being transported within the biofouling on pleasure craft hulls. In the Netherlands, they are applied by only a selection of boat owners and harbour masters, either due to practical considerations (e.g. costs, lack of dry-docking space in some marinas) or because of a lack of awareness. Increased awareness of these recommendations is therefore expected to reduce the risk that aquatic invasive species are distributed by pleasure crafts. The cost will be borne by the shipping companies or private vessel owners (for fishing and recreational vessels). Costs associated with hull fouling management measures are	considerable reduction in the arrival of high-risk vessels between 2015, when national anti-fouling guidelines were voluntary, and after 2018, when they had entered into force as legislation (Hayes et al., 2019). Considerable uncertainty exists with respect to the management of environmental risks associated with in-water cleaning technologies (Scianni & Georgiades, 2019).

		again not specific to *B. proboscidea* but refer to all marine NIS. Traditional dry-docking costs hundreds of thousands of euros, and the cost of reapplying a new layer of antifouling amounts to half the total cost. Indicatively, typical in-water cleaning of a 180-200 m container vessel conducted by companies in the US east coast would take approximately two days for an entire hull; cost of application in the range of €17-43k. Emerging IWC systems are currently available in only a few locations worldwide, and are more costly than traditional methods (Zabin et al., 2016). The cost to marina owners of establishing a biosecure treatment facility for the disposal of hull fouling material was estimated to be at least £45-50K in the UK (DEFRA, 2012).	
	P3. Managing the pathway "Bilge water" There are currently no legal acts or official guidance for the prevention of NIS transportation with bilge water. Potential mitigation measures (Fletcher *et al.*, 2017): • Restrictions on the location of discharge • retention of bilge water for subsequent disposal • treatment of bilge water prior to discharge • awareness campaigns to boaters • industry codes of practice	Requiring discharge within the source region seems the most practical option available, with no associated costs and minimal logistic constraints. Desirable vessel operator behaviours can be promoted through awareness campaigns to educate boaters about possible risks from bilge water, similar to the "Clean, Drain, Dry" campaigns that are widely implemented in the USA in relation to aquatic (freshwater) hitchhikers (http://stopaquatichitchhikers.org/aboutus/).	**Low** These methods are not currently practiced in the marine environment and are based on expert opinion.

	P4. Managing the pathway "Contaminant on Aquaculture" Council Regulation (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed to minimise the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. According to the regulation, all aquaculture operators who intend to introduce an alien species or translocate a locally absent species must first apply for a permit from the competent authority of the Member State where the transfer will take place. The Regulation specifies the information to be provided by the applicant and the type of assessment that the competent authority must perform before granting the permit. The ICES Code of Practice on the Introductions and Transfers of Marine Organisms (ICES, 2005)	The bivalves *Crassostrea gigas* and *Ruditapes philippinarum* listed in Annex IV of Council Regulation 708/2007 constitute an exception and can be moved without any risk assessment or quarantine; also the regulation does not apply to movements of locally absent species within the Member States "except for cases where, on the basis of scientific advice, there are grounds for foreseeing environmental threats due to the translocation, Art. 2 para. 2." While there are no records of *B. proboscidea* on clams *R. philippinarum* (Radashevsky, pers.comm), the polychaete is regularly associated with both wild and cultivated populations of *C. gigas* in Japan, Australia, South Africa (see Simon & Sato-Okoshi, 2015 for a review) as well as with wild *C. gigas* in the vicinity of culture plots in the Netherlands (Kerckhof & Faasse, 2014; Wijnhoven et al., 2017) and a cultured oyster in France (Radashevsky et al., 2019). Oyster transfers within and between EU States are extensive (Muehlbauer et al., 2014) and could facilitate spread of *B. proboscidea* in the RA area. However, additional local, national or European (e.g. Natura2000 related) regulations may apply that limit the translocation possibilities of species like *C. gigas* and *R. philippinarum* throughout Europe. Shellfish transports to Dutch outer waters (the Oosterschelde) are, for example, only allowed with a permit that can be obtained when following a strict management and control system aimed at minimizing the risk of introducing nuisance species (Bleker,	**Medium** When properly implemented, this suit of measures can be effective in detecting and preventing introductions and spread of alien species. There are, however, indications that best practice procedures are not always adhered to for shellfish movements throughout the RA area (e.g., Haydar & Wolff, 2011; Theodorou et al., 2011).

	recommends the procedure for introduced or transferred species which are part of current commercial practice. The procedure states clearly that: a) all products should originate from sources in areas that meet current codes, such as the OIE International Aquatic Animal Health Code or equivalent EU directives. b) if required, there should be inspection, disinfection, quarantine or destruction of the introduced organisms and transfer material (e.g., transport water, packing material, and containers) based on OIE or EU directives. Lastly, using hatchery-produced seed reduces risk of introduction and spread through stock/seed transfers.	2012; Gittenberger et al., 2017). Stricter regulations also apply in the trilateral Wadden Sea area, particularly in relation to Natura2000 sites, where conservation objectives may be threatened (WG-AS & Gittenberger, 2018). Restrictions on transfers based on the risk associated with the source areas is an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk NIS are available. The cost of sourcing, obtaining the necessary permission and harvesting or transferring alternative bivalve (seed) populations will vary with production and current/future harvesting location and practice and may be considerable for the producer – this mainly applies to countries with less strict intra-state shellfish transport regulations. Non-compliance with regulations may be reduced with more effective policing/monitoring and stronger sanctions but would have a high associated cost. Visual inspections of the thoroughness afforded during aquaculture operations are largely ineffective for the detection of *B. proboscidea* eggs and larval stages on	

		shellfish, as they are often hidden in other species' borrows or crevices of the shell (Simon *et al.*, 2009; 2010) Shellfish hatcheries enable the industry to produce seed consistently but at a much higher cost (Kamermans, 2008).	
Methods to achieve eradication[6]	Theoretically, eradication may be possible for localised, newly established populations at low densities with limited dispersal capabilities or no local recruitment (Delaney & Leung, 2010; Ojaveer *et al.*, 2015; Grosholz & Ruiz, 2002). This would require an early warning system, monitoring efforts and a removal programme.	Due to difficulties in detection (small, tube-dwelling as well as boring species) and identification but also due to *B. proboscidea*'s capabilities for both long-range dispersal and almost continuous local recruitment throughout the year, the eradication of the species in the wild is considered unlikely. Additionally, local eradication would require ongoing, long-term, regular interventions due to the ongoing risk of spread from well-established, surrounding populations. Effectiveness will also depend on the existence of a clear 'action plan' to follow in case of early interception and well publicised guidance on what to do if individuals are found. In particular, such guidance would need to include reporting and removal instructions and would need to be publicised to specific groups (shellfish growers, marina owners and users) likely to encounter individuals.	**Medium** Eradication campaigns in the wild have not been attempted anywhere in the invaded range. Additionally, it is widely acknowledged in the literature that eradications in the marine environment are highly unlikely to succeed, unless species are detected very early and a rapid response plan is in place.
	E1. Early warning systems / awareness raising The species requires specialized taxonomic expertise for its identification, such that awareness raising and early	An effective platform for such knowledge exchange can be the network INVASIVESNET, which aims to facilitate greater understanding and improved management of invasive alien species (IAS) and biological invasions globally by linking new	**Medium** It is difficult to measure the long-term effectiveness of these programmes,

	warning systems are better designed with a focus on the training of professionals who are likely to encounter/collect it during the course of monitoring or other marine survey activities, as well as potentially affected stakeholders (i.e. shellfish growers).	and existing networks of interested stakeholders (Lucy *et al.*, 2016). Other suitable platforms for awareness and knowledge exchange are Accreditation and Quality Control schemes, like the "North East Atlantic Marine Biological Analytical Quality Control (NMBAQC) Scheme: Benthic Invertebrates and Particle Size Components", which operates in the UK and regularly brings together experts from environmental consultancy companies and competent monitoring authorities. This platform is considered most effective for early detection of *Boccardia proboscidea* due to the high professional level of experts and the constant sampling provided by participating companies all round Europe. Cost-efficiency is high, as these schemes are already in place but there may be some limitations associated with data accessibility from commercial projects.	the recent influx of records from northern Europe however indicates that as taxonomic experts become increasingly aware of the species, its reliable identification and detection will aid early warning.
	E2. Monitoring and early detection Within the framework of the Marine Strategy Framework Directive (MSFD) and in order to reach Good Environmental Status targets with reference to Descriptor D2 (Non-indigenous species), most EU states are already designing or implementing national/regional NIS-targeted monitoring	NIS-targeted monitoring through national or local programmes has detected *B. proboscidea* at low densities in ports, marinas and among oysters in the UK and the Netherlands (Hatton & Pearce, 2013; Kakkonen *et al.*, 2019; Wijnhoven *et al.*, 2017) and in intertidal mussel beds in France (Spilmont *et al.*, 2018), however, the "age" of these populations/introduction events cannot be ascertained. The efficacy of these methods in detecting even early life stages of *B. proboscidea* will be high at monitoring hotspots for new introduction events, particularly if eDNA	**Medium**

	programs (see ICES (2017) for national reports in France, Germany, Sweden, Denmark, Norway; see Minchin (2014) for Ireland). Monitoring should focus on introduction hotspots (e.g., ports, marinas, aquaculture plots) and specific natural hotspots (e.g., oyster reefs, wild mussel beds) on a yearly basis (ICES, 2017). In the case of *B. proboscidea* in particular, organically enriched habitats (in the vicinity of introduction hotspots) should also constitute monitoring targets for the detection of development of locally abundant populations. Molecular tools and DNA barcoding of zooplankton samples may represent a further early detection approach, that could be extended to ballast water and bilge water monitoring (Zaiko *et al.,* 2015; Fletcher *et al*., 2017). eDNA methodologies are currently being actively explored by a number of EU States (ICES	methodologies are implemented for ballast and bilge water; natural dispersal from existing populations, however, leads to a very large endangered area, which cannot be fully covered by monitoring programs. Involving the general public and citizen scientists can greatly increase monitoring effort, it is however considered unfeasible in the case of *B. proboscidea* (see E1 above). With regards to the cost of monitoring, an indicative estimate comes from Denmark, where the cost of a proposed hotspot monitoring program for all marine NIS (covering 13 port and three areas with discharges of cooling water) was estimated at approximately €125k for the period 2015-2017 (Andersen et al., 2014). In the UK, a broad initial estimate of monitoring costs for MSFD D2 alone (considering that existing or new surveys for other descriptors will also contribute to the monitoring of NIS) suggests that they would be less than €961k over 10 years (DEFRA, 2012) with an additional €100k for drafting legislation and guidance. These are general monitoring costs and not specific to *B. proboscidea*.	

	WGITMO, 2017) as tools for the detection of NIS in introduction hotspot water samples and ballast water, and can greatly aid early detection - even of larval stages (Darling *et al.*, 2017).		
	E3. Removal program - Physical/mechanical removal - application of chemical agents (e.g. copper sulfate (CuSO4), chlorine, formaldehyde, calcium hydroxide (lime), iodine, acetic acid, or a hydrated lime solution (Morse *et al.*, 2015 and references therein)).	Due to the tube-dwelling, interstitial or boring life-style of the species, mechanical removal would also entail removal of the associated substrate, something unrealistic, infeasible and unadvised. Chemical control is usually not recommended because it is not species-specific and can have deleterious impacts on native fauna (Grosholz & Ruiz, 2002). However, when applied over localised areas/populations, the drawbacks of chemical control may be acceptable if eradication of the species is achieved, as evidenced by the successful eradication of *Caulerpa taxifolia* with chlorine treatment in California (Anderson et al., 2005; Williams & Grosholz, 2008). In the case of *B. proboscidea*, intentional chemical treatment for the purpose of eradication has not been attempted, the biogenic reefs formed by the species in Argentina however disappeared on more than one occasion from sewage impacted sites due to the chlorination of untreated sewage effluent by the local water authorities. This also resulted in the disappearance of all macro-organisms from the area within a 3km radius (Jaubet *et al.*, 2013). Several months later *B. proboscidea* reefs re-appeared in the area., the species however was already widespread in the region. The	**Medium** The degree of effectiveness of various chemical agents has been demonstrated in shellfish culture systems for different spionid species (with the exception of chlorine – see comment in adjacent box) and rarely resulted in complete eradication of infestations. Outside culture systems, where survival/fitness of cultivated species is not a consideration, there is evidence that chlorination can

		application of chemical agents may require changes in legislation, while its long-term effectiveness may be compromised by recruitment from neighbouring populations. Nevertheless, in the case of early detection of a relatively isolated population, chemical treatment offers the best chance of eradication.	be an effective eradication measure.
Methods to achieve management[7]	**Management of populations in the wild** Continuous monitoring in introduction and natural hotspots, as well as organically enriched (e.g. sewage impacted) locations. Management of nutrient and organic matter discharges at invaded habitats that are likely to foster dense populations can effectively prevent the development of high level, ecosystem effects (i.e. biogenic reefs and associated biodiversity loss). Local depletion efforts if deemed necessary (see removal methods in section E3)	Considering that, for *B. proboscidea*, the most critical factor for the manifestation of impacts on biodiversity and ecosystem services is the development of dense populations, perhaps the most effective management measure (outside culture systems) would be to maintain organic matter content in water and sediments at levels that do not favour outbreaks of opportunistic polychaete species and ensure Good Environmental Status (values will be highly regionally specific and recommended nutrient and organic inputs will depend on local hydrodynamic conditions). Such efforts are already in place within the RA area to ensure compliance with MSFD mandates and standards and any additional monitoring can build on existing national MSFD monitoring schemes. Successful depletion efforts can also prevent local populations from acting as source populations for further spread (Duncombe & Therriault, 2017). The limitations of the removal methods apply also to population control efforts.	**Medium**

	Mitigation of impacts in culture systems A number of methods have been tested and used to combat mudworm infestations of cultured bivalves and gastropods (e.g. Handlinger *et al*., 2004; Nell, 2007; Haupt *et al*., 2012; Morse *et al*., 2015). These include: • manual cleaning and jet-blasting with seawater • exposure to freshwater • exposure to heated seawater • immersion in brine followed by air drying • planting at higher shore levels/intertidally • exposure to air • exposure to phyco-derived compounds (e.g. Simon *et al.*, 2010) • treatment with chemical agents (abalone) The list provided above is not exhaustive but covers possible mitigation measures with the greatest prospect of practical use.	Spionid infestations of *C. gigas* can be avoided/reduced by growing oysters above extreme low water neap and 0.5 m above the mud substratum (Handley & Bergquist, 1994). A variety of chemical agents against *Boccardia knoxi* infesting the blacklip abalone *Haliotis rubra* were tested by Handlinger *et al.* (2004), who demonstrated that such treatments had a minimal effect on mud worms in shell burrows or were harmful to abalone at doses high enough to kill the worms. The best treatment for mud worm in abalone was found to be simple air-drying of stock.	**Medium** The effectiveness of these methods is demonstrated for different mudworm species but generally a reasonable measure of success is expected, particularly if *B. proboscidea* infestations are more prevalent on the outer surface of the shells.

	Moreover, using hatchery-produced seed may reduce the risk of spread of *B. proboscidea* with stock transfers.	Shellfish hatcheries enable the industry to produce seed consistently but at a much higher cost (Kamermans, 2008).	

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.

- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion.This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2[1]

Name of organism: *Schizoporella japonica*

Author(s) of the assessment:

Jack Sewell & Christine A. Wood, The Marine Biological Association, UK

Risk Assessment Area: The risk assessment area is the territory of the European Union, excluding the outermost regions.

Peer review 1: *Marika Galanidi, Dr, Dokuz Eylul University, Institute of Marine Sciences and Technology, Izmir, Turkey*

Peer review 2: *Argyro Zenetos, Dr, Research Director, Hellenic Centre for Marine Research, Greece*

Date of completion: *31/10/19*

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968).

Contents

SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

Response:
The taxonomic family, order and class to which the species belongs:
Bryozoa (Phylum); Gymnolaemata (Class); Cheilostomatida (Order); Flustrina (Suborder); Schizoporelloidea (Superfamily); Schizoporellidae (Family); *Schizoporella* (Genus)

The scientific name and author of the species, as well as a list of the most common synonym names:
Schizoporella japonica Ortmann, 1890

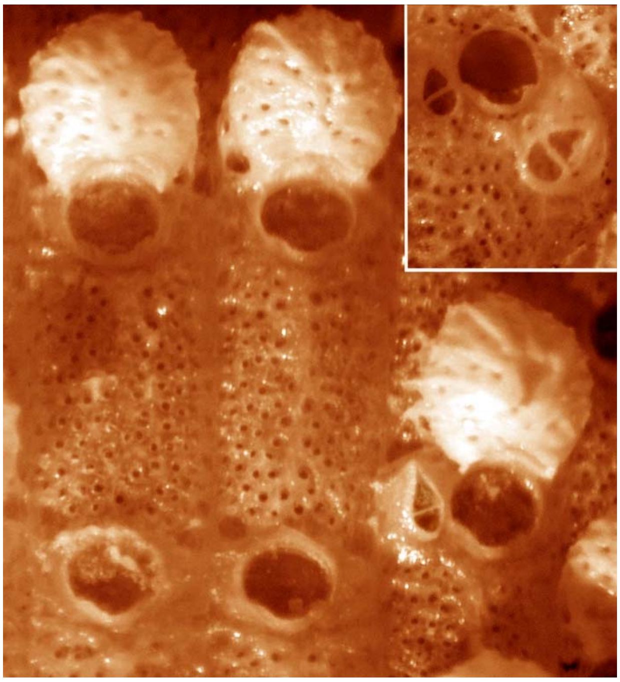
Synonyms:
Schizoporella unicornis var. *japonica* Ortmann, 1890
The name used to describe when first identified in Japan. Also used in some papers describing distribution in North America. It was later elevated to separate species status as *Schizoporella japonica* (Bock 2015). Records of *S. unicornis* from Australia are considered as likely to be *S. japonica* also (Ryland et al 2015).

Names used in commerce (if any):
None known

A list of the most common subspecies, lower taxa, varieties, breeds or hybrids:
None known

Developed, encrusting colony on marina equipment, showing unilaminar and bilaminar growths Photo: John Bishop, MBA

Bilaminar, orange growth Photo: Christine Wood, MBA



Close up of zooids, showing white ovicells, perforated frontal wall, orifice and sinus shape, and avicularia. Photo: John Bishop, MBA

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;

native species, potential misidentification and mis-targeting

Response: It is possible that colonies may bear a superficial, passing resemblance to encrusting sponges, colonial ascidians or encrusting algal growths, however the presence of uniformly-sized, regularly, continuously arranged zooid 'cells' (visible with the naked eye) make bryozoan colonies distinctive from any of these.

There are a large number of orange encrusting bryozoan species, which occur in the risk assessment area, both native and introduced, that, to the naked eye, show similarities to *S. japonica* e.g. *Cryptosula pallasiana*, *Oshurkovia littoralis, Escharoides coccinea, Schizobrachiella sanguinea, Smittina spp.,Turbicellepora magnicostata* (all native to the North-East Atlantic). *S. japonica* has a distinctive bright orange colouration, although this may be more or less apparent depending on the age and condition of colonies. Use of a hand lens to look at the shape of the zooidal structures will eliminate many of these confusion species. However, microscopic investigation will be required in most cases, and for a conclusive identification of a suspected new introduction SEM imaging or DNA analysis will be necessary.

The most difficult to distinguish are other species within the genus *Schizoporella,* all members of the genus have rectangular or polygonal zooids, regular perforations in the frontal wall, a D-shaped orifice with a U or V- shaped sinus, prominent globular ovicells, and single or paired avicularia to the side of the orifice (see figure in A-1). However, there is 'extensive confusion in identifying species within the genus *Schizoporella* (IUCN 2019, Dick et al, 2005; Porter, 2012), which has led to the historic misidentification and reporting of species in the genus. Special care should therefore be taken in observing and identifying specimens, with reference to relevant keys and scientific papers e.g. Hayward & Ryland (1995); Ryland et al 2014.

The following species are the most likely to cause identification confusion:

S. unicornis (Johnston in Wood, 1844) (Native to North-East Atlantic)

S. dunkeri (Reuss, 1848) (Native to North-East Atlantic)

S. errata (Waters, 1878) (Native to East Atlantic, from Iberian coast and Mediterranean, Invasive in Australasia)

S. pseudoerrata (Soule & Chaney, 1995) (Distribution uncertain not native to North-East Atlantic)

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: A risk assessment has been undertaken for Norway and it has been considered a High risk, with high chance of invasiveness, with a small ecological impact and high level of uncertainty (Oug et al 2019).

A4. Where is the organism native?

including the following elements:

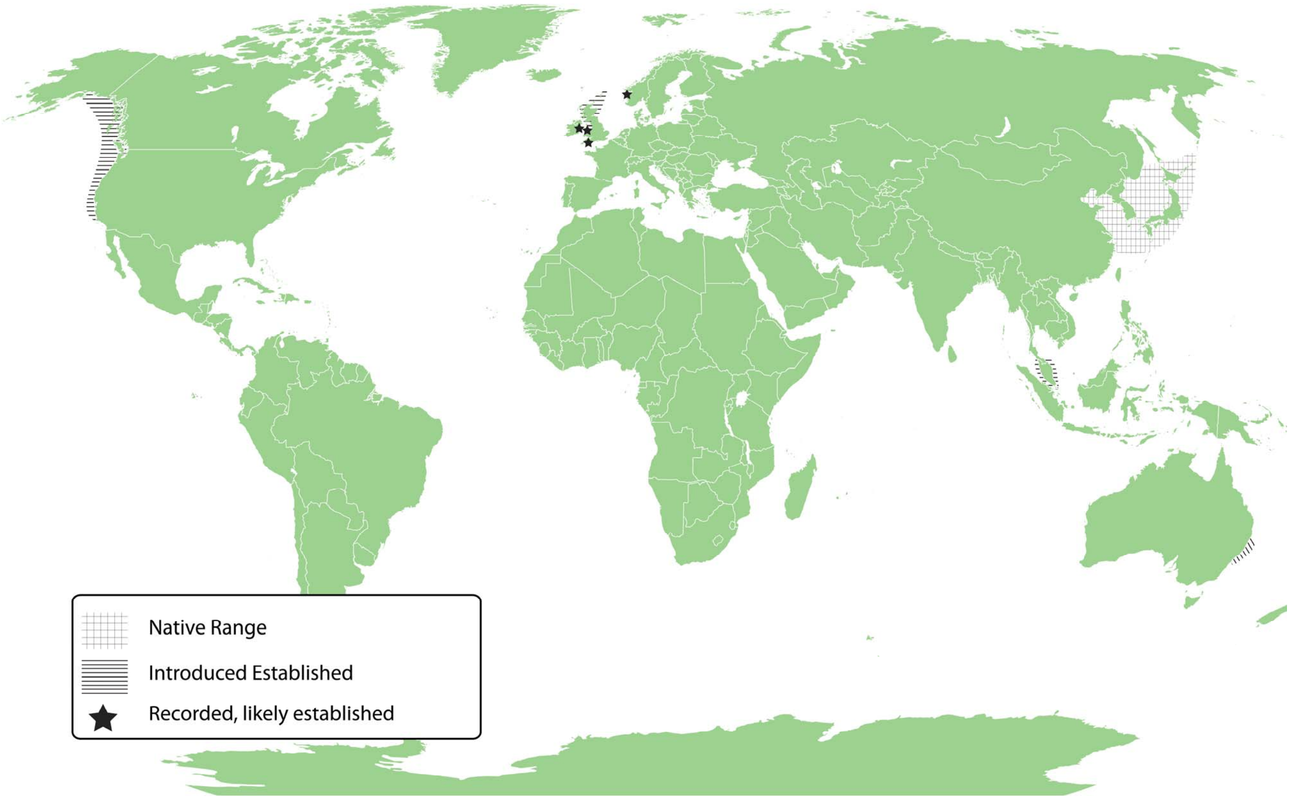
- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: The north-west Pacific from China to Japan. It is considered very unlikely that *S. japonica* will spread naturally into the risk assessment area from its native range.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

*Schizoporella japonica (*described as *S. unicornis)* (Dick et al 2005) was introduced to the north-eastern Pacific on oysters from Japan (Powell, 1970). It has been reported along the Pacific coast of North America from Alaska to California. It is likely also be present in Australia where *S. unicornis* (possibly *S. japonica*) was reported in 1975 following imports of Pacific oysters (Dick et al 2005). The precise introduced range is currently unknown, as it is commonly misidentified as *S. unicornis* or *S. errata* (Dick et al 2005; Treibergs 2012). Reproducing, introduced populations have been identified in Malaysia (Taylor & Tan 2015).



Current suspected distribution of *Schizoporella japonica* based on information taken from: Ocean Biographic Information System (OBIS); Global Biodiversity Information Facility (GBIF); Loxton et al (2017); Dick et al (2005).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded and established occurrences.

Response (6a) Recorded:

Marine regions:

North-east Atlantic Ocean

Marine subregions:

Greater North Sea, Celtic Seas

Response (6b) Established:

Marine regions:

North-east Atlantic Ocean

Marine subregions:

Greater North Sea; Celtic Seas

Assertion is based on data collated from the Global Biodiversity Information Facility (GBIF) and the Ocean Biogeographic Information System, as well as the literature studied in the preparation of this report, particularly Loxton et al (2017), which provides a comprehensive and recent overview. Also observations of Celtic Seas populations made by the authors (unpublished). Due to historic misidentification, there is a possibility that the range may be greater than currently known.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (A7a): Regarding *S. japonica*, temperature and salinity are considered the most important climatic variables in limiting establishment for the species. Based on current conditions, salinity is within the known tolerance range of 15-36ppt (Powell 1970, Loxton 2014) throughout the Greater North Sea, Celtic Seas, Bay of Biscay and northern Iberian Coast and Bay of Biscay, as well as parts of the Black Sea, (see Map Appendix 1). Salinity in the and southern Iberian Coast is considered to be too high and in the Baltic Sea, and north-west Black Sea salinity is considered to be below the level which the species is known to tolerate. In areas of the Mediterranean with lower levels of salinity – for example the Thau Lagoon, France and Venice Lagoon, Italy –as well as the lower Iberian coast, salinity levels reach a maximum of 37ppt, which is only marginally higher than the known survivable level of 36ppt and seasonally, temporarily drops to tolerable levels. Given the adaptability of the species and lack of detailed research into the tolerances of the species and regional variants, introduction and establishment in these areas should not be completely discounted.

Temperature range in which the species is known to reproduce is wide and records from Malaysia (Taylor and Tan 2015) suggest a highest temperature tolerance of 30ºC, whilst records from Alaska and elsewhere suggest a lowest temperature tolerance of -1.4 ºC (CABI 2019). With this in mind, February and August sea temperatures throughout the EU are within the tolerable range for the species, suggesting salinity may play the most important role in restricting the spread of the species to The Greater North Sea, Celtic Seas, and Bay of Biscay and (northern part of) Iberian Coast (see Appendix 1).

Response (A7b): Based on future air temperature warming scenarios RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065).). Bruno et al (2018) predict temperate marine areas will experience a maximum SST increase of 2.6°C under BAU (Business as usual) RCP 8.5 scenario. In the event that this occurs, the maximum temperature for survival will be exceeded in some parts of the Mediterranean (southern Italy, areas around the Balearics and areas outside the RAA). As presence in these areas is likely to already be limited by salinity levels in the area, this does not represent a significant change of potential habitable area under these scenarios.

Projections of sea surface salinity (SSS) under future climate conditions can be much more variable, depending largely on the model used for predictions (Pushpadas et al 2015; Schrum et al 2016; Thiébault & Moatti 2016). In the Mediterranean Sea, a progressively higher SSS is however generally projected with values ranging from 0.06 psu to 1 psu over the next 100 years, depending on the scenario employed (Thiébault & Moatti 2016), while freshening of the North Sea, the Baltic Sea as well as the Iberian coast (Jordà et al 2017) in the order of -0.1 to -0.6 psu may be anticipated under different scenarios. Thus, in the two regions where salinity was acting as a limiting factor for establishment, the direction of change will further limit the potential for *S. japonica* to become established, (i.e. even higher salinities in the Mediterranean and even lower salinities in the Baltic Sea). A projected freshening of the Iberian coast may result in a higher likelihood of establishment along the entire Iberian coast (see maps in Appendix 1).

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden,

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): United Kingdom, Ireland. UK - First record Plymouth 2009; Holyhead, Wales 2010; Orkney, Scotland 2011; Scottish coast 2013; Blyth, NE England 2016. Ireland - Greystones Marina 2015.

Response (8b): United Kingdom. First established population noted in Holyhead, Wales 2010.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): For full explanation of expected potential range for establishment see 7a. Member states are: Belgium (offshore), Netherlands (offshore), France, Ireland, Portugal, Spain, UK, Denmark, Germany, Sweden.

Response (9b): Predicted warming scenarios will not cause an increase that is likely to exceed the tolerable temperature at a member state scale. Nor will states not currently considered suitable become suitable as sea temperature increases. It is not anticipated that predicted warming scenarios will alter the member states in which the species might be able to become established.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: *S. japonica* is a competitor for space and is known to inhibit the growth of adjacent species. It has proved very capable of colonising and dominating natural and man-made habitat and competitively excluding or overgrowing native species, in particular where it has invaded through its North American range (Dick et al 2005). However, it is suggested that it may be a poor invader of previously occupied space (Sutherland, 1978).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Response: North-east Atlantic Ocean - Greater North Sea and, Celtic Seas.

Populations in the Northern Isles of Scotland have become a dominant member of fouling communities on artificial structures and have started to colonize natural substrate in the wild, competing for space with native species (Nall et al 2015, Loxton et al 2017). In Anglesey, North Wales, the species dominated fouling communities in Holyhead marina, appearing to competitively exclude other species following clearance of the invasive tunicate *Didemnum vexillum* (Ryland et al 2014). In Plymouth, England it similarly initially dominated the fouling in a newly constructed marina and spread to two further nearby marinas (unpublished observations by authors).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Response: United Kingdom – Scottish Northern Isles; Anglesey, North Wales; Plymouth, England.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response: No information has been found.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided)”.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[2] and the provided key to pathways[3].
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9

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[2] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%%20pathways%20Final.pdf

[3] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

Pathway name: **Transport: Contaminant - Contaminant on animals**

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	unintentional	**CONFIDENCE**	medium

Response: The primary introduction of *S. japonica* outside its native range is believed to have been as a result of accidental transportation with the commercially grown Pacific oyster *Magallana (*was *Crassostrea) gigas* when the species was exported during the early twentieth century from Japan to the west coast of North America (Loxton et al 2017) . Records of *S. unicornis* (considered likely to be *S. japonica*) from Australia were reported following the introduction of Pacific oysters (Dick et al 2005). Although bivalve introductions are now carefully regulated to minimize the risk of importation of contaminated stock, introduction to member states via movements of shellfish from outside the RAA is possible. Additionally, introduction of contaminated stock to neighboring countries with different levels of control in place is feasible.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	unlikely	**CONFIDENCE**	medium

Response: *Schizoporella japonica* is known to attach to a wide range of substrates and structures likely to occur in close proximity to bivalves in culture (e.g. aquaculture equipment, maintenance vessels and structures, and natural seabed) (Collin et al 2015). This represents a source of propagules which might be transported into the RAA. There is substantial evidence to suggest that the species will attach and grow readily on living bivalves and empty shells, including oysters (see examples in Loxton et al 2017). The species is hermaphroditic and reproduction is likely to take place year-round. Larvae are brooded, with a short larval phase of a few hours before settlement (Ryland et al 2014). These traits make contamination of shellfish stored in close proximity to colonies of *S. japonica* likely. Once settlement takes place, individuals reproduce and spread by budding asexually to form encrusting sheets. Therefore, the successful settlement, development and growth of an individual on a transporting organism can result in a high level of propagule potential. However, EC regulation 708/2007 (EC 2007) aims to reduce the impact of introduced alien species from aquaculture and requires processes to be undertaken to ameliorate the potential environmental damage caused by such introductions. This should theoretically include measures to reduce the potential for 'hitchhiking' species to be introduced and may require careful treatment, quarantine and other processes before stock can be released into the wild. It is however unlikely that such stringent measures will be applied to movements of stock within member states and where species, normally native to or established in a member state are introduced. Where this is the case, it is difficult to evaluate whether or not management practices will be utilized that would effectively reduce the likelihood of introduction. It is considered that the measures currently in place mean that large numbers will not be introduced over the course of a year.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Based on previous introductions, *S. japonica* has a very high potential to survive transportation attached to live shellfish (Dick et al 2005; Loxton et al 2017; Powell et al 1970; Ryland et al 2014)). The survivable temperature range is high (-1.4 - 30ºC) (Loxton et al 2017, CABI 2019). Reproduction through asexual budding and colony growth is likely provided conditions are favourable and sexual reproduction (which takes place year round) is also likely.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	moderately likely	**CONFIDENCE**	medium

Response: EC regulation 708/2007 (EC 2007) aims to reduce the impact of introduced alien species for aquaculture and requires processes to be undertaken to ameliorate the potential environmental damage caused by such introductions. This should theoretically include measures to reduce the potential for 'hitchhiking' species to be introduced and may require careful treatment, quarantine and other processes before stock can be released into the wild. It is however unlikely that such stringent measures will be applied to movements of stock where species, normally native to or established in a member state are introduced. For example, exemptions exist regarding the introduction of the Pacific oyster *Magallana gigas* (listed as *Crassostrea*) which has been implicated as a potential pathway of introduction of *S. japonica* in North America and Australia. Where this is the case, it is difficult to evaluate whether or not management practices will be utilized that would effectively reduce the likelihood of introduction.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Ancestrula (first colonising zooid) measure 350-400 x ~300 µm (Ryland et al 2014) and at this stage are extremely difficult to detect. A single ancestrula can develop and reproduce asexually to form colonies of hermaphroditic, reproductive individuals. Developed colonies are easier to detect measuring several centimetres across and being bright orange. However, confusions over identification and the large number of taxonomically similar native species reduce the likelihood that the species will be identified and intercepted except by experts, unless regular eDNA monitoring of aquaculture sites is instigated.

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	Unlikely	**CONFIDENCE**	medium

Response: Based on the biological traits of the species and past examples of introductions globally by this particular pathway, introduction is considered possible. Legislation restricting the import and release of alien species for aquaculture (EC 2007) mean the likelihood of introduction by this pathway is reduced significantly by non-species specific management measures. The risk of introduction when attached to species already native to or established or species illegally introduced in the RAA is less certain and for this reason, the score 'unlikely' has been allocated.

End of pathway assessment, repeat Qu. 1.2 to 1.7 as necessary using separate identifier.

Pathway name: **Transport: Stowaway - Ship/boat hull fouling**

Qu. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: *Schizoporella japonica* is known to attach to and colonize a range of man-made structures including the hulls of commercial vessels, ferries and recreational vessels (Collin et al 2015; Ryland et al 2014; Bishop et al 2015). It is also known to colonize marina pontoons, fenders and other structures likely to occur in the vicinity of vessels. Global shipping and recreational boat travel takes place between areas from which the species is known and ports, harbours and marinas within the RAA. Hull fouling has been implicated in the spread of the species globally and to the UK, Ireland and Norway (Ashton et al 2014; Loxton et al 2017). The discontinuous UK distribution and the current concentration of records in and in close proximity to structures associated with recreational and commercial shipping, coupled with the species' low natural dispersal potential is further evidence to suggest that hull fouling is a primary vector.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	likely	**CONFIDENCE**	high

Response: *Schizoporella japonica* is known to attach to and colonize a range of man-made structures including the hulls of commercial vessels, ferries and recreational vessels (Collin et al 2015; Ryland et al 2014; Bishop et al 2015). It is also known to colonize marina pontoons, fenders and other structures likely to occur in the vicinity of vessels. Populations are therefore likely to exist in proximity to and therefore may spread to vessels which may travel to the RAA. Global shipping and recreational boat travel takes place between areas from which the species is known and ports, harbours and marinas within the RAA on a regular basis.

The species is hermaphroditic and sexual reproduction is likely to take place year-round. Larvae are brooded, with a short larval phase of a few hours before settlement, (Ryland et al 2014) giving time for the ciliated larvae to move between structures and moored vessels. These traits make attachment to the hulls of vessels moored in close proximity to colonies of *S. japonica* likely. Once settlement takes place, individuals reproduce and spread by budding asexually to form sheets. Therefore, the successful settlement, development and growth of an individual on man-made objects such as a vessel hull can result in a high level of propagule potential.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Based on previous introductions, *S. japonica* has a very high potential to survive transportation attached to vessel hulls (Ashton et al. 2014; Loxton et al. 2017). The survivable temperature range is high (-1.4 - -30ºC) (Loxton et al 2017, CABI 2019). Reproduction through asexual budding and colony growth is likely provided conditions are favourable and sexual reproduction (which takes place year round) is also likely.

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	likely	**CONFIDENCE**	Medium

Response: Hull cleaning is an often practiced method of defouling ship and boat hulls and has the potential to physically remove colonies of *S. japonica* (although colony fragments are likely to remain), which would in turn reduce the risk of introduction. However the practice is not legally required before vessels enter the RAA and can be financially costly making it very likely that vessels traveling between contaminated and uncontaminated marinas and ports will not have been treated.

The authors were unable to find information about the ability of *S. japonica* to resist antifouling treatments, however it should be noted that in the congener *S. errata,* Cu (copper) based antifouling coatings on boat hulls can prevent growth of *S. errata* and stop its spread to new locations (Piola and Johnston 2006). Although (as with physical hull cleaning), antifouling is not currently a legal requirement, there is potential that treatments with biocidal compounds may prove an effective method of controlling fouling and reduce the likelihood of spread.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Ancestrula measure 350-400 x ~300 µm (Ryland et al 2014) and at this stage are extremely difficult to detect. A single ancestrula can develop and reproduce asexually to form colonies of hermaphroditic, reproductive individuals. Developed colonies are easier to detect measuring several centimetres across and being bright orange. However, confusions over identification and the large number of taxonomically similar native species reduce the likelihood that the species will be identified and intercepted except by experts.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Introduction to the RAA (UK and Ireland) has already taken place within in the last 10 years, presumably via this pathway. Introduced populations currently exist in the neighboring state of Norway. From here commercial and recreational shipping, as well as passenger ferries travel regularly to destinations within the RAA. The various biological traits described in previous sections, as well as the nature of the currently colonized sites mean that transfer to these vessels and those travelling from other sites internationally is highly likely.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	Very likely	**CONFIDENCE**	High

Response: Introductions have occurred to the UK and Ireland within the last 10 years. Most records were from locations associated with shipping and recreational boating activity suggesting shipping is a major vector (Loxton et al 2017; Ryland et al 2014). It has not yet been possible to ascertain the source population of these invasions, it is therefore difficult to say with certainty whether the route by which the species arrived in the UK would be likely to reoccur or that it would be a potential route that would impact other member states. The population now present in Norway (Porter et al 2015) does represent a potential source population with a high likelihood of transportation into the European Union.

Introductions via shellfish is considered less likely due to relevant legislation and restrictions in place regarding the movement of shellfish from outside the EU. This may be possible for species already present in the union for example *Mytilus edulis* and *Magallana gigas* (listed as *Crassostrea*).

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)

- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	likely	**CONFIDENCE**	medium

Response: *S. japonica* is primarily considered to be a cold-water species (Loxton et al 2017) however records of reproducing populations from Malaysia (Taylor and Tan 2015) suggest a far higher temperature tolerance with temperatures of up to 30 ^{0}C being suitable for growth and reproduction. It is therefore considered likely that temperature increases predicted under both RCP 2.6 and RCP 4.5 would be unlikely to change the potential for the species to be introduced by the pathways described.

It is possible that with melting sea ice caused by increasing temperatures, new Arctic trade routes may open up, increasing the likelihood that non-native species might be introduced by shipping (Miller & Ruiz 2014). If this were to happen, the established, non-native Alaskan population of *S. japonica*, present in and around harbours but also in wild habitats – might become a source population, being transported by commercial vessels. Such change would certainly increase the likelihood of introduction to the North Sea and Celtic Seas in particular, but additional regions may be vulnerable, depending on the nature of novel shipping routes.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[4] and the provided key to pathways[5].
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name: **Transport: Contaminant - Contaminant on animals**

Qu. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: For introduction summary see **1.2a.** If infected bivalves are grown in open systems or laid in wild growing sites, the invading, hitchhiking species will have ‘entered into the environment’.

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

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[4] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf

[5] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	moderately likely	**CONFIDENCE**	medium

Response: *Schizoporella japonica* is known to attach to a wide range of substrates, including shellfish and finfish aquaculture equipment as well as a range of other structures (Collin et al 2015). This has the potential to place a source of propagules in close proximity to bivalves in culture, which might be transported into the RAA. There is substantial evidence to suggest that the species will attach and grow readily on living bivalves, including oysters (see examples in Loxton et al 2017). The species is hermaphroditic and reproduction is likely to take place year-round. Larvae are brooded, with a short larval phase of a few hours before settlement (Ryland et al 2014). These traits make contamination of shellfish stored in close proximity to colonies of *S. japonica* likely. Once settlement takes place, individuals reproduce and spread by budding asexually to form sheets Therefore, the successful settlement, development and growth of an individual on a transporting organism can result in a high level of propagule potential.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Ancestrula measure 350-400 x ~300 µm (Ryland et al 2014) and at this stage are extremely difficult to detect. A single ancestrula can develop and reproduce asexually to form colonies of hermaphroditic, reproductive individuals. Developed colonies are easier to detect measuring several centimetres across and being bright orange. However, confusions over identification and the large number of taxonomically similar native species reduce the likelihood that the species will be identified and intercepted except by experts.

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very likely	**CONFIDENCE**	high

Response: *S. japonica* reproduces year round (Ryland et al 2014; Loxton 2017; Treiburgs 2012) and therefore has the potential to spread and become established at any time of the year. It has been suggested that the ability of *S. japonica* to reproduce and remain active during colder periods may provide a competitive edge over some species, which become dormant over winter, suggesting this might be the most appropriate time for establishment (Loxton et al 2017; Ryland et al 2014).

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Non-feeding ciliated larvae are brooded and released from ovicells , these larvae persist for only a few hours (Loxton et al 2017), but during this time are able to transfer from their host to an adjacent surface. Multiple hard ssubstrates have proved a suitable habitat for settlement and growth, including equipment and structures associated with aquaculture and natural hard substrate, such as shell, rock and boulders (see review in Loxton et al 2017), which are all likely to be found in the vicinity of bivalve culture sites.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	likely	**CONFIDENCE**	high

Response: although transport to the RAA via this pathway is consider unlikely due to legal constraints, if transportation does occur, arrival would be very likely due to the nature of bivalve culture operations, which usually occur in open systems or in wild settings, providing a proliferation of suitable habitat within natural dispersal distance. As discussed, the year-round reproductive potential of *S. japonica* gives it the potential to become established at any time of the year through release of propagules.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Pathway name: **Transport: Stowaway - Ship/boat hull fouling**

Qu. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: *Schizoporella japonica* is known to attach to and colonize a range of man-made structures including the hulls of commercial vessels, ferries and recreational vessels (Collin et al 2015; Ryland et al 2014; Bishop et al 2015). The apparently wide, discontinuous distribution around the UK, with records primarily from within marinas (Bishop et al 2015) suggests a connection with recreational vessels and may represent multiple introductions via this pathway or, more likely multiple examples of human mediated spread from sites within the RAA. It is also known to colonize marina pontoons, fenders and other structures likely to occur in the vicinity of vessels. Global shipping and recreational boat travel takes place between areas from which the species is known and ports, harbours and marinas within the RAA. Hull fouling has been implicated in the spread of the species globally and to the UK, Ireland and Norway (Ashton et al 2014; Loxton et al 2017). The current concentration of records in and in close proximity to structures associated with recreational and commercial shipping, coupled with the species' low natural dispersal potential is further evidence to suggest that hull fouling is a primary vector.

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	moderately likely	**CONFIDENCE**	medium

Response: *Schizoporella japonica* is known to attach to and colonize a range of man-made structures including the hulls of commercial vessels, ferries and recreational vessels (Collin et al 2015; Ryland et al 2014; Bishop et al 2015). It is also known to colonize marina pontoons, fenders and other structures likely to occur in the vicinity of vessels, Global shipping and recreational boat travel takes place between areas from which the species is known and ports, harbours and marinas within the RAA on a regular basis.

The current concentration of records in and in close proximity to structures associated with recreational and commercial shipping (Loxton et al 2017), suggest a potential source of propagules. The species is hermaphroditic and reproduction is likely to take place year-round. Larvae are brooded, with a short larval phase of a few hours before settlement, (Ryland et al 2014) giving time for the ciliated larvae to move between structures and moored vessels. These traits make attachment to the hulls of vessels moored in close proximity to colonies of *S. japonica* likely. Once settlement takes place, individuals reproduce and spread by budding asexually to form sheets. Therefore, the successful settlement, development and growth of an individual on man-made object such as a vessel hull can result in a high level of propagule potential.

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Ancestrula measure 350-400 x ~300 µm (Ryland et al 2014) and at this stage are extremely difficult to detect. A single ancestrula can develop and reproduce asexually to form colonies of hermaphroditic, reproductive individuals. Developed colonies are easier to detect measuring several centimetres across and being bright orange. However, confusions over identification and the large number of taxonomically similar native species reduce the likelihood that the species will be identified and intercepted except by experts. Mobile larvae are microscopic and therefore very unlikely to be detected without specialist sampling, equipment and expertise.

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	likely	**CONFIDENCE**	high

Response: Response: *S. japonica* reproduces year round (Ryland et al 2014; Loxton 2017; Treiburgs 2012) and therefore has the potential to spread and become established at any time of the year. It has been suggested that the ability of *S. japonica* to reproduce and remain active during colder periods may provide a competitive edge over some species, which become dormant over winter, suggesting this might be the optimal time for establishment (Loxton et al 2017; Hayward et al 2014). Conversely this is the time of year when recreational boat users are less active and the likelihood of arrival by this particular pathway might be slightly reduced.

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Non-feeding ciliated larvae are brooded and released from ovicells, these larvae persist for only a few hours (Loxton et al 2017), but during this time are able to transfer from vessel hull to an adjacent surface. Multiple hard substrates have proved a suitable habitat for settlement and growth, including equipment and structures associated with commercial and recreational shipping and natural hard substrate, such as shell, rock and boulders (see review in Loxton et al 2017), which are all likely to be found in the vicinity of many marinas and ports. In some instances recreational vessels might moor in natural areas, adjacent to suitable natural substrate or a secondary structure such as a chain, rope or buoy, where propagules might be deposited leading to growth of new colonies.

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Once transported into the RAA via this pathway, the nature of recreational and commercial shipping activities means that arrival in the natural environment is very likely. Shipping takes place in open systems, putting fouling colonies of *S. japonica* in close proximity to suitable habitat. Once in a suitable place propagules are able to travel the short distance to nearby suitable habitat. Such habitat might be a fully natural substrate or man-made object, which might provide a ‘stepping stone’ for the species to colonize adjacent natural habitat.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	Very likely (North-East Atlantic) Likely (Black Sea)	**CONFIDENCE**	High (North-East Atlantic) Medium

	Unlikely (Iberian coast, Mediterranean)		(Black Sea) Medium (Iberian coast, Mediterranean)

Response: *Schizoporella japonica* is known to attach to and colonize a range of man-made structures including the hulls of commercial vessels, ferries and recreational vessels (Collin et al 2015; Ryland et al 2014; Bishop et al 2015). It is also known to colonize marina pontoons, fenders and other structures likely to occur in the vicinity of vessels. Global shipping and recreational boat travel takes place between areas from which the species is known and ports, harbours and marinas within the RAA.

Hull fouling has been implicated in the spread of the species globally and to the UK, Ireland and Norway (Ashton et al. 2014; Loxton et al. 2017). The current concentration of records in and in close proximity to structures associated with recreational and commercial shipping, coupled with the species' low natural dispersal potential is further evidence to suggest that is a primary vector. The potential for *S. japonica* to enter the environment from imported shellfish once transported is considered very likely. However the likelihood of introduction via this particular pathway is not clear due to the legal barriers described in section 2.2.

An additional consideration is that, due to the sessile nature of adults, in order for entry to take place after introduction via the pathways discussed, conditions must be suitable for reproduction. This would mean that Entry might be unlikely in the Mediterranean and along the southern Iberian coastline, which are currently outside the known tolerable salinity range and at the top end of the temperature tolerance for the species. Hull-fouling colonies would need to survive travel across the Mediterranean basin – which would expose colonies to unfavourable conditions - in order to arrive in the Black Sea via the hull fouling pathway. It is therefore considered less likely that entry will take place in the Black Sea, although the ability of the species to survive these less favourable conditions for short periods of time is not well studied and confidence is therefore considered to be medium.

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	Very likely (North-East Atlantic) Likely (Black Sea) Unlikely (Mediterranean)	**CONFIDENCE**	High (North-East Atlantic) Low (Black Sea) Low (Mediterranean)

Response: Based on future warming scenarios RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). The maximum temperature for survival is likely to be exceeded in some parts of the Mediterranean (southern Italy, areas around the Balearics and areas outside the RAA). As entry potential in these areas is likely to already be limited by salinity levels in the area, this does not represent a significant change under these scenarios (see maps in Appendix 1). There is potential that these changes may increase the likelihood of mortality during transport through the Mediterranean, however the extent to which this will reduce the risk of entry further in the Black Sea cannot be predicted with certainty based on current available knowledge. The potential reduced salinity in the Iberian oast region (see A7b) suggests that this region may become habitable under future scenarios.

Other than the differences described above, the likelihood of entry and confidence are the same for future scenarios as described in 2.8.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism's current distribution)?

RESPONSE	very likely	**CONFIDENCE**	High (northern)- Medium (southern)

Response: It is considered very likely that *S. japonica* will be able to become established if introduced throughout the Greater North Sea region, Celtic Seas, Bay of Biscay and the northern Iberian coast. It is also possible that establishment may occur in the Black Sea although introduction is considered far less likely. Salinity is within the 15 – 36ppt range, in which *S. japonica* is able to survive and reproduce (Loxton et al 2017) throughout this area, whilst the southern Iberian coast and Mediterranean exhibit higher salinities than this, reducing the likelihood of establishment if introduced. Levels of salinity in the Baltic region fall below those currently known to support the species, making establishment in this region unlikely.

Temperatures throughout the RAA are within the known tolerable range for *S. japonica* to survive and reproduce, considered to be -1.4 to 30ºC (Loxton et al 2017, CABI 2019). This suggests that temperature is unlikely to be a factor limiting the establishment of the species. It has been observed that reproduction takes place throughout the year under colder conditions (7-15ºC) (Treibergs 2012; Ryland et al 2014; Loxton 2014) and establishment patterns seem to show a faster rate of establishment in colder regions of the UK as opposed to warmer ones (Loxton et al 2017). The authors were unable to find information about reproductive rates under warmer conditions, but it is possible, based on evidence from the UK that establishment may be slower in southern, warmer regions and that temperature may play a role.

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	ubiquitous	**CONFIDENCE**	high

Response: *Schizoporella japonica* is able to grow successfully on a range of hard substrates, including man-made objects - such as sea defences, pontoons, jetties and vessels – as well as natural hard substrates - such as shell, loose rock and bed rock (Loxton et al 2017). It is able to colonies floating objects as well as shallow subtidal and intertidal habitat. Suitable habitat is therefore considered ubiquitous throughout the RAA.

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	N/A	**CONFIDENCE**	high

Response: *S. japonica* is not dependent on any other organism at any stage of its lifecycle.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	likely	**CONFIDENCE**	medium

Response: *S. japonica* displays a number of traits, which makes it a highly competitive species, capable of colonizing areas quickly once introduced. It broods larvae (Dick et al 2005) providing protection during early stages in development. Once released, larvae travel only a short distance before settlement occurs and individuals reproduce asexually by budding, forming extensive colonies enabling rapid colonization of areas. The species has been shown to outcompete native bryozoan species in Alaska (Dick et al 2005). It is capable of overgrowing a number of native species, including mussels and other bryozoan species, causing mortality in some cases (Treibergs 2012; Macleod et al 2016). It has also been suggested that the ability of *S. japonica* to reproduce and develop colonies in cold conditions may provide a competitive edge against species which become dormant over winter, enabling space to be occupied. Efforts to clear pontoons in Holyhead Marina, Wales, UK led to colonization of cleared substrate by the species (Ryland et al 2014) demonstrating its ability to competitively colonize newly cleared areas. Colonies persisted in these areas beyond 18 months (Christine Wood and John Bishop observation reported in Loxton et al 2017) further illustrating the potential for the species to persist once settled and become established. Similarly a new marina in Plymouth was rapidly colonized in 2012 and the population is still present in 2019 (personal observation by author).

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	likely	**CONFIDENCE**	Low

Response: Little information could be found to suggest that establishment by *S. japonica* would be limited by predators, parasites or pathogens. However, new recruits may be vulnerable to predation by predatory and grazing invertebrates immediately after metamorphosis and attachment to the substrate. For example, predation by flatworms of embryos and larvae still in ovicells has been observed in the field (Gordon 1972; Treibergs 2012). The extent to which this might impact establishment is not clear, but patterns of previous successful establishments (for example Loxton et al 2017; Ryland et al 2014 and Treibergs 2012) suggest that at least in some areas, this is not likely to be a factor preventing establishment throughout the whole RAA.

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	very likely	**CONFIDENCE**	high

Response: At present, no or little legislation exists, which requires marina or harbour structures or equipment to be cleared of fouling or treated to prevent fouling taking place. Whilst some vessel owners do carry out cleaning and treatment of vessels, which may reduce introduction, establishment is unlikely to be impaired. Moreover, management efforts to eradicate another fouling, invasive species *Didemnum vexillum* in Holyhead Marine, Wales, UK actually seemed to promote the settlement and establishment of *S. japonica* (Ryland et al 2014) suggesting other measures to manage fouling species may have the unintended impact of increasing likelihood of establishment.

Establishment within aquaculture sites is less likely due to the legislation previously mentioned in 1.5a.

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	moderately likely	**CONFIDENCE**	medium

Response: Management efforts to eradicate another fouling, invasive species *Didemnum vexillum* in Holyhead Marine, Wales, UK actually seemed to promote the settlement and establishment of *S. japonica* (Ryland et al 2014) suggesting other measures to manage fouling species may have the unintended impact of increasing likelihood of establishment.

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	likely	**CONFIDENCE**	High

Response: The difficulties distinguishing the species from other bryozoans and the small size at settlement – *S. japonica* ancestrula measure 350-400 x ~300 µm (Ryland et al 2014) – make it highly likely that targeted physical removal measures will be of limited success. Physical removal of colonies by scraping would probably fragment the brittle encrustations, which could lead to increased spread as any fragments not captured could be dispersed, potentially releasing larvae elsewhere. Moreover, the ability of *S. japonica* to rapidly colonize newly cleared areas, as demonstrated, following the removal of the invasive ascidian *Didemnum vexillum* from a marina in North Wales (Ryland et al 2014) means that more general clearance might actually benefit the settlement and competitive dominance of the species.

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very likely	**CONFIDENCE**	high

Response: Once introduced in the RAA and entry in the environment has taken place, the biological characteristics of the species make establishment very likely, particularly in colder regions where reproduction is known to be continuous throughout the year, resulting in a regular flow of propagules from colonized areas.

Colonies begin with a single, sexually produced zooid, which buds asexually to produce sheets - which may be unilamellar or bilamellar depending on conditions and substrate – of hermaphroditic individuals (Loxton et al 2017). Larvae are brooded in external brood chambers (ovicells) and non-feeding, ciliated (swimming) larvae are released. Once released, larvae have a short dispersal period of a few hours, after which attachment and metamorphosis occurs. This adaptation facilitates colonization locally and explains the rapid appearance of dense colonies, but limits the range of dispersal unless mediated by an additional vector (likely human) once settled. When settlement does not occur after 24 hours (due to lack of available substrate), laboratory studies have shown that settlement does not take place and larvae are prone to die (Treibergs 2012).

Suitable habitat for settlement is ubiquitous throughout the RAA and a diverse range of solid substrates are suitable for the growth of colonies (Collin et al 2015), meaning that habitat availability is unlikely to be a limiting factor in the establishment of the species.

The wide range of temperatures (-1.4 - 30ºC) under which reproduction has been observed in wild colonies (Loxton 2014; Taylor and Tan 2015, CABI 2019) suggests that settlement and establishment would be possible throughout the RAA, however salinity in the Mediterranean, Baltic and southern Iberian coastline falls outside the currently known tolerance window of 15-36ppt. Malaysian records (Taylor and Tan 2015) suggests a maximum tolerance of 30ºC based on average ambient sea water temperature locally. However, the majority of information regarding the reproductive ability of the species is from cooler areas and the authors could not find further details of reproductive ability in conditions exceeding 19ºC. The ability of the species to become established in warmer areas is therefore less certain.

Studies have shown that *S. jap*onica is sensitive to high levels of turbidity, with presence showing a negative correlation with turbidity in colonization studies and with a zero probability of colonization at values exceeding 30 NTU (Treibergs 2012) which may further restrict colonization in some areas.

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	moderately likely	**CONFIDENCE**	high

Response: *Schizoporella japonica* is very unusual among the bryozoa in its ability to generate multiple ovicells on an individual zooid. It has been proposed that this may be an aberration caused by pollution (Powell 1970) or a naturally occurring modification (Loxton et al 2017), which may enhance reproductive output in less favourable conditions or in founding populations.

The wide temperature range (-1.4 - 30ºC) which can be tolerated by the species (Loxton et al 2017; Loxton 2014; Taylor and Tan 2015, CABI 2019) and ability to colonize a wide variety of biotic, abiotic and man-made substrates (Loxton et al 2017) demonstrates adaptability to extremely variable conditions. As does its demonstrated ability to rapidly colonize newly cleared areas (Ryland et al 2014).

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	likely	**CONFIDENCE**	medium

Response: There is no reason to suppose that low genetic diversity would inhibit the ability of the species to become established. *S. japonica* is characterized as being hermaphroditic and having brooded, ciliated, coronate larva, which do not spend long in the water column and therefore travel only short distances before attachment and metamorphosis (Loxton et al 2017). Other species of bryozoan sharing this liffe-history trait (as opposed to releasing cyphonautes larvae, which spend a greater amount of time in the plankton and disperse over greater distances) tend to exhibit low genetic differentiation (Watts and Thorpe 2006). Its ability to reproduce asexually by budding means that individual animals can grow to cover large areas, and potentially spread to other areas via fragmentation.

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	likely	**CONFIDENCE**	high

Response: Considering that *S. japonica* is able to produce propagules year round (Loxton et al 2017) and that there is regular movement of recreational and commercial vessels between sites populated and other areas within the RAA, it is likely that introductions will continue to be transported into new areas within the RAA or to be re-introduced in areas multiple times. The pattern of records in Plymouth, UK suggests multiple introductions following an initial record from 2009 (Loxton et al 2017 and author observations).

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very likely	**CONFIDENCE**	high

Response: *Schizoporella japonica* has become established within northern parts of the RAA (North of Scotland, North Wales, South-West England) and neighboring Norway (Loxton et al 2017), there are large parts of the RAA with very similar climatic conditions and habitat to these areas, throughout the Greater North Sea and Celtic Seas regions. Sea temperature and habitat availability are extremely similar in the known range of *S. japonica* on the west coast of the America (Alaska to California) (Dick et al 2005), to the Celtic Seas, Greater North Sea, Iberian Coast and Bay of Biscay. Salinity is also similar, although south of Bilbao, Spain and into the Mediterranean region levels exceed those in the current known USA range and known tolerances of the species, suggesting that establishment would be less likely in these areas. It is important to note however that given uncertainty over the taxonomy of the species, the full native and introduced range of the species globally may not be completely known. The more recent records from Malaysia (Taylor and Tan 2015) illustrate this well, as the records were from an area where the known ambient water temperature exceeded that known from the current introduced range by approximately 10°C. Illustrating the need for caution when predicting the potential range of a species that is so little known and apparently tolerant of such a wide range of conditions.

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.
With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	likely	**CONFIDENCE**	low

Response: Based on known range and tolerances, it is likely that the area in which *S. japonica* might become established will be limited primarily by salinity, which is higher south of Bilbao, Spain than the salinity levels in the known current distributional range. The entire RAA is currently within the known tolerable temperature window for the species and will remain so even under future climate change scenarios (RCP 2.6 and RCP 4.5). However, as discussed in 3.13, patterns of establishment so far in the RAA suggest that colder conditions are preferable, possibly due to an increased competitive advantage over ‘winter dormant’ species (Loxton et al 2017). If this is the case, warming seas may decrease the range at which this competitive edge is attained. However, not enough is understood about the parameters under which this might operate to make any accurate estimates.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic "spread" via release or escape ("jump-dispersal"), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minor	**CONFIDENCE**	medium

Response:

Natural larval dispersal

S. japonica is hermaphroditic and produces brooded, ciliated, coronate larva, which do not spend long in the water column and therefore travel only short distances before attachment and metamorphosis (Loxton et al 2017). It is therefore likely that natural spread throughout the RAA will be slow and may be restricted by 'barriers' such as stretches of unsuitable habitat or unfavourable current flows (Watts & Thorpe 2006).

Rafting

Schizoporella japonica is known to attach to debris and flotsam and to travel long distances by this method. For example, following the Japanese Tsunami in 2011, colonies of living *S. japonica* (alive with embryos) were identified on objects originating in Japan and found on the Hawaiian Islands and North American coast after traversing the Pacific Ocean (and Carlton 2018). It is therefor possible that colonies may develop on natural objects which may become flotsam, providing a pathway of spread.

Hitchhiking on mobile species

No examples could be found of *S. japonica* colonizing mobile fauna specifically, however similar species are known to grow on the exoskeletons of crabs (e.g. *Hyas areneus, Maja squinado, Cancer pagurus*), many species of which exist in the RAA and move or migrate significant distances throughout the RAA. The ability of *S. japonica* to colonies such a wide range of substrates, including shellfish, suggests that spread by this vector is possible.

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	major	**CONFIDENCE**	medium

Response: Due to the limited natural dispersal potential of the species, it is believed that spread will be largely dependent on human facilitation. Many of these methods are described by Loxton et al (2017).

Transport: Stowaway - Ship/boat hull fouling (Movement of recreational vessels, ferries and commercial vessels)

Many of the existing Atlantic records of *S. japonica* are in or around recreational and commercial boating facilities (Loxton et al 2017), suggesting that the likelihood of propagules settling on vessels visiting these areas is high. There are many well used sailing routes connecting colonized and not-yet-colonized sites around the RAA (Loxton et al 2017), and given the year-round larval release exhibited by the species, the chances of settlement during busy times of the year is very high. Additionally, there are numerous passenger ferry and shipping routes connecting Scotland with the rest of the RAA providing additional modes of spread.

Transport: Stowaway Machinery/ Equipment (Movement of equipment associated with aquaculture and renewable energy structures)

As the demand for renewable energy increases, there are increasing numbers of marine structures associated with the industry, particularly on the Scottish coast. Nall (2015) studied such structures and found that they provided an ideal habitat for colonization by *S. japonica*. It was also discovered that many structures associated with offshore renewables are stored for significant periods of time in ports known to hold populations of *S. japonica* before being transported elsewhere for deployment (Loxton et al 2017). Such practices present a clear potential vector for spreading fouling species such as *S. japonica.*

Transport: Contaminant - Contaminant on animals (Transfer of live shellfish)

The primary introduction of *S. japonica* outside its native range is believed to have been as a result of accidental transportation with the commercially grown oyster *Magallana (*was *Crassostrea) gigas* when the species was exported during the early twentieth century from Japan to the west coast of North America (Loxton et al 2017) . Records of *S. unicornis* (considered likely to be) from Australia were reported following the introduction of Pacific oysters (Dick et al 2005). Although bivalve introductions are now carefully regulated to minimize the risk of importation of contaminated stock, introduction to member states via movements of shellfish between sites is possible. Additionally, introduction of contaminated stock to neighboring countries with different levels of control in place is feasible.

Shellfish, such as oysters mussels, scallops, cockles and clams are grown in open systems and transported between sites for on-growing throughout the RAA, currently movement within and between states is possible in most cases and there is certainly potential for colonized shellfish to become a vector of spread for organisms like *S. japonica*. Many operations notably take place on the coast of Scotland and North Wales.

Stowaway: Attachment to floating debris (corridor?)

S. japonica is known to attach to debris and flotsam and to travel long distances by this method, for example following the Japanese Tsunami in 2011, colonies of living *(alive with embryos)* were identified on objects originating in Japan and found on the Hawaiian Islands and North America after traversing the Pacific Ocean (McCuller and Carlton 2018). It is therefore possible that colonies may develop on drifting plastic, lost fishing equipment and other man-made objects which may become flotsam, providing a pathway of spread within the RAA. With an increase in drifting marine litter, this potential vector is becoming increasingly prevalent.

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.
- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name: **Transport: Stowaway - Ship/boat hull fouling (Movement of recreational vessels, ferries and commercial vessels)**

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: Many of the existing Atlantic records of *S. japonica* are in or around recreational and commercial boating facilities (Loxton et al 2017), suggesting that the likelihood of propagules settling on vessels visiting these areas is high. There are many well used sailing routes connecting colonized and not-yet-colonized sites around the RAA (Loxton et al 2017) and given the year-round larval release exhibited by the species, the chances of settlement during busy times of the year is very high. Additionally, there are numerous passenger ferry and shipping routes connecting Scotland with the rest of the RAA providing additional modes of spread.

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very likely	**CONFIDENCE**	high

Response: *Schizoporella japonica* is known to attach to and colonize a range of man-made structures including the hulls of commercial vessels, ferries and recreational vessels (Collin et al 2015; Ryland et al 2014; Bishop et al 2015). It is also known to colonize marina pontoons, fenders and other structures likely to occur in the vicinity of vessels. Global shipping and recreational boat travel takes place between areas from which the species is known and ports, harbours and marinas within the RAA on a regular basis.

The species is present in a number of busy port and marina areas (Scottish Northern Isles, wider Scotland and northern England, North Wales, Plymouth and East coast of Ireland) (Loxton et al. 2017), which host passenger ferries, commercial vessels and recreational vessels travelling throughout the RAA on a regular basis. These movements provide ample opportunity for transportation within the RAA from existing invaded populations.

The species is hermaphroditic and reproduction is likely to take place year-round. Larvae are brooded, with a short larval phase of a few hours before settlement, (Ryland et al 2014) giving time for the ciliated larvae to move between structures and moored vessels, these traits make attachment to the hulls of vessels moored in close proximity to colonies of *S. japonica* likely. Once settlement takes place, individuals reproduce and spread by budding asexually to form sheets..

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Based on previous introductions, S. japonica has a very high potential to survive transportation attached to vessel hulls (Ashton et al 2014; Loxton et al 2017). The survivable temperature range is high (-1.4 - 30ºC) (Loxton et al 2017, CABI 2019). Reproduction through asexual budding and colony growth is likely provided conditions are favourable and sexual reproduction (which takes place year round) is also likely.

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	likely	**CONFIDENCE**	high

Response: Hull cleaning is an often practiced method of defouling ship hulls and has the potential to physically remove colonies of *S. jap*onica, which would in turn reduce the risk of spread. However the practice is not legally required, particularly for vessels moving within the RAA and can be financially costly making it very likely that vessels traveling between contaminated and uncontaminated marinas and ports will not have been treated. In addition, complete removal of colonies is unlikely due to their brittleness, small fragments may remain or be dispersed.

The authors were unable to find information about the ability of *S. japonica* to resist antifouling treatments, however it should be noted that in the congener *S. errata,* Cu (copper) based antifouling coatings on boat hulls can prevent growth of *S. errata* and stop its spread to new locations (Piola and Johnston 2006). Although (as with physical hull cleaning), antifouling is not currently a legal requirement, there is potential that treatments with biocidal compounds may prove an effective method of controlling fouling and reduce the likelihood of spread.

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Ancestrula measure 350-400 x ~300 µm (Ryland et al 2014) and at this stage are extremely difficult to detect. A single ancestrula can develop and reproduce asexually to form colonies of hermaphroditic, reproductive individuals. Developed colonies are easier to detect measuring several centimetres across and being bright orange. However, confusions over identification and the large number of taxonomically similar native species reduce the likelihood that the species will be identified and intercepted except by experts. The use of regular eDNA monitoring in marinas and harbours could improve early detection.

Qu. 4.8a How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very likely	**CONFIDENCE**	high

Response: Non-feeding ciliated larvae are brooded and released from ovicells, these larvae persist for only a few hours (Loxton et al 2017) and Maximum 24 hours (Treibergs 2012), but during this time are able to transfer from their host to an adjacent surface. Multiple hard substrates have proved a suitable habitat for settlement and growth, including equipment and structures associated with aquaculture and natural hard substrates, such as shell, rock and boulders (see review in Loxton et al 2017), which are all likely to be found in the vicinity of ports, harbours and marinas.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	moderately	**CONFIDENCE**	medium

Response: Following the introduction of *S. japonica* in the UK in or not long before 2009, the species has spread widely throughout the UK and into Ireland. The discontinuous nature of the current known range coupled with its presence in locations associated with recreational sailing (Loxton et al 2017), suggests that this vector has been used effectively to spread within the area rapidly, expanding its range by more than 900miles in 10 years. It is not unreasonable to suppose that this rate of spread could continue throughout the RAA. It is also important to consider that populations may already exist in the RAA or on vessels moving within the RAA, undetected or misidentified, due to the cryptic nature of the species and therefore, rate of spread may be higher than anticipated.

Pathway name: **Transport: Stowaway Machinery/ Equipment (Movement of equipment associated with aquaculture and renewable energy structures)**

Qu. 4.3b. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: As the demand for renewable energy increases, there are increasing numbers of marine structures associated with the industry, particularly on the Scottish coast. Nall (2015) studied such structures and found that they provided an ideal habitat for colonization by *S. japonica*. It was also discovered that many structures associated with offshore renewables are stored for significant periods of time in ports known to hold populations of *S. japonica* before being transported elsewhere for deployment (Loxton et al 2017). Such practices present a clear potential vector for spreading fouling species such as *S. japonica.*

Qu. 4.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very likely	**CONFIDENCE**	high

Response: *Schizoporella japonica* is known to attach to and colonize a range of man-made structures including structures associated with wind and wave power generation (Collin et al 2015; Ryland et al 2014; Bishop et al 2015), predominantly not coated in antifouling paints (Nall 2015). It is also known to colonize marina pontoons, fenders and other structures likely to occur in the vicinity of this gear when stored before being towed to a deployment site, from which further spread or natural settlement might occur.

The species is hermaphroditic and reproduction is likely to take place year-round. Larvae are brooded, with a short larval phase of a few hours before settlement (Ryland et al 2014), giving time for the ciliated larvae to move between structures and moored vessels. These traits make attachment to the hulls of vessels moored in close proximity to colonies of *S. japonica* likely. Once settlement takes place, individuals reproduce and spread by budding asexually to form sheets.

Qu. 4.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	likely	**CONFIDENCE**	high

Response: Based on previous introductions, *S. japonica* has a very high potential to survive transportation attached to equipment and machinery that remains in the sea (Ashton et al 2014; Loxton et al 2017). The survivable temperature range is high (-1.4 - 30°C) (Loxton et al 2017, CABI 2019). Reproduction through asexual budding and colony growth is likely provided conditions are favourable and sexual reproduction (which takes place year round) is also likely.

Qu. 4.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	likely	**CONFIDENCE**	medium

Response: The authors were unable to find information about the ability of *S. japonica* to resist antifouling treatments, however it should be noted that in the congener *S. errata,* Cu (copper) based antifouling coatings on boat hulls can prevent growth of *S. errata* and stop its spread to new locations (Piola and Johnston 2006). Many marine renewable energy and aquaculture structures are not treated with antifouling paints (Nall 2015), however, treatments with biocidal compounds may prove an effective method of controlling fouling and reduce the likelihood of spread.

There are very limited management measures in place currently to reduce the spread of non-native species when moving previously deployed equipment within the RAA.

Qu. 4.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Ancestrula measure 350-400 x ~300 μm (Ryland et al 2014) and at this stage are extremely difficult to detect. A single ancestrula can develop and reproduce asexually to form colonies of hermaphroditic, reproductive individuals. Developed colonies are easier to detect measuring several centimetres across and being bright orange. However, confusions over identification and the large number of taxonomically similar native species reduce the likelihood that the species will be identified and intercepted except by experts. The methods required to check equipment which remains submerged even when not in use, are costly (diving) or not necessarily able to observe sufficient detail to identify bryozoans to species level (ROVs). The use of regular eDNA monitoring at relevant sites may improve detection.

Qu. 4.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	likely	**CONFIDENCE**	medium

Response: Non-feeding ciliated larvae are brooded and released from ovicells, these larvae persist for only a few hours (Loxton et al 2017), with a maximum of 24 hours (Treibergs 2012), but during this time are able to transfer from their parent to an adjacent surface. Multiple hard substrates have proved a suitable habitat for settlement and growth, including equipment and structures associated with aquaculture and natural hard substrates, such as shell, rock and boulders (see review in Loxton et al 2017), which are all likely to be found in the vicinity of ports and harbours used to store equipment.

If equipment is to be deployed on the seabed, intertidally or in the vicinity of vessels or natural hard substrates, propagules should be able to colonize these neighbouring areas easily. However for equipment deployed further out at sea, off the seabed, transfer may be secondary via service vessels, debris and transfer to a suitable habitat may not occur directly.

Qu. 4.9b. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	slowly	**CONFIDENCE**	medium

Response: There is very little information about the rate of species spread based on this particular vector, however, it is likely that spread will be sporadic as objects are transported to dock after long periods at sea and then redeployed. It is likely that the distance of travel will be relatively low and that rate of spread would be very much object specific.

Pathway name: **Transport: Contaminant - Contaminant on animals (Transfer of live shellfish)**

Qu. 4.3c. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: The primary introduction of *S. japonica* outside its native range is believed to have been as a result of accidental transportation with the commercially grown oyster *Magallana (*was *Crassostrea) gigas* when the species was exported during the early twentieth century from Japan to the west coast of North America (Loxton et al 2017) . Records of *S. unicornis* (considered likely to be *S. japonica*) from Australia were reported following the introduction of Pacific oysters (Dick et al 2005). Although bivalve introductions are now carefully regulated to minimize the risk of importation of contaminated stock, introduction to member states via movements of shellfish between sites is possible. Additionally, introduction of contaminated stock to neighbouring countries with different levels of control in place is feasible.

Shellfish, such as oysters, mussels and scallops are grown in open systems and transported between sites for on-growing throughout the RAA Currently movement within and between states is possible in most cases and there is certainly potential for colonized shellfish to become a vector of spread for organisms like *S. japonica*. Many such operations notably take place on the coast of Scotland and North Wales.

Qu. 4.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	likely	**CONFIDENCE**	high

Response: *Schizoporella japonica* is known to attach to a wide range of substrates, including shellfish and finfish aquaculture equipment as well as a range of other structures (Collin et al 2015). This has the potential to place a source of propagules in close proximity to bivalves in culture, which might be transported within the RAA. There is substantial evidence to suggest that the species will attach and grow readily on living bivalves, including oysters (see examples in Loxton et al 2017). The species is hermaphroditic and reproduction is likely to take place year-round. Larvae are brooded, with a short larval phase of a few hours before settlement (Ryland et al 2014). These traits make contamination of shellfish stored in close proximity to colonies of *S. japonica* likely. Once settlement takes place, individuals reproduce and spread by budding asexually to form sheets. Therefore, the successful settlement, development and growth of an individual on a transporting organism can result in a high level of propagule potential.

Qu. 4.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	likely	**CONFIDENCE**	High

Response: Based on previous introductions, *S. japonica* has a very high potential to survive transportation attached to live shellfish (Dick et al 2005; Loxton et al 2017; Powell et al 1970; Ryland et al 2014)). The survivable temperature range is high (-1.4 - 30ºC) (Loxton et al 2017, CABI 2019). Reproduction through asexual budding and colony growth is likely provided conditions are favourable and sexual reproduction (which takes place year round) is also likely.

Qu. 4.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	moderately likely	**CONFIDENCE**	medium

Response: EC regulation 708/2007 (EC 2007) aims to reduce the impact of introduced alien species ffrom aquaculture and requires processes to be undertaken to ameliorate the potential environmental damage caused by such introductions. This should theoretically include measures to reduce the potential for 'hitchhiking' species to be introduced and may require careful treatment, quarantine and other processes before stock can be released into the wild. It is however unlikely that such stringent measures will be applied to movements of stock within and between member states and where species, normally native to or established in a member state are introduced. Where this is the case, it is difficult to evaluate whether or not management practices will be utilized that would effectively reduce the likelihood of introduction.

Qu. 4.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Ancestrula measure 350-400 x ~300 µm (Ryland et al 2014) and at this stage are extremely difficult to detect. A single ancestrula can develop and reproduce asexually to form colonies of hermaphroditic, reproductive individuals. Developed colonies are easier to detect measuring several centimetres across and being bright orange. However, confusions over identification and the large number of taxonomically similar native species reduce the likelihood that the species will be identified and intercepted except by experts. The use of regular eDNA monitoring at aquaculture sites may reduce the risk of spread.

Qu. 4.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	likely	**CONFIDENCE**	high

Response: Non-feeding ciliated larvae are brooded and released from ovicells, these larvae persist for only a few hours (Loxton et al 2017), but during this time are able to transfer from their host to an adjacent surface. Multiple hard substrates have proved a suitable habitat for settlement and growth, including equipment and structures associated with aquaculture and natural hard substrates, such as shell, rock and boulders (see review in Loxton et al 2017), which are all likely to be found in the vicinity of bivalve culture sites.

Qu. 4.9c. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	moderately	**CONFIDENCE**	medium

Response: Movement of shellfish is controlled and moderated between member states, however, practices such as the transfer of mussel seed to growing beds and the movement of oysters to optimize growing conditions, may result in the spread of *S. japonica* attached to equipment, bivalves and associated substrate. Mussel seed might be transported hundreds of kilometers and spread over a wide area, providing optimal conditions for *S. japonica* to grow and form large colonies resulting in natural spread from multiple points. Such activity would be likely to spread *S. japonica* rapidly, but again sporadically and in a discontinuous fashion. The current status of populations in the USA, 40 years after introduction and spread via this vector, suggests that over time distribution will become less discontinuous as introduced populations spread naturally (albeit slowly) and connect.

Pathway name: **Stowaway/ corridor: Attachment to floating anthropogenic debris**

Qu. 4.3d. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	unintentional	**CONFIDENCE**	high

Response: *Schizoporella japonica* is known to attach to debris and flotsam and to travel long distances by this method,. For example following the Japanese Tsunami in 2011, colonies of living *S. japonica* (alive with embryos) were identified on objects originating in Japan and found on the Hawaiian Islands and North American coast after traversing the Pacific Ocean (and Carlton 2018). It is therefor possible that colonies may develop on drifting plastic, lost fishing equipment and other man-made objects, which may become flotsam, providing a pathway of spread within the RAA. With an increase in drifting marine litter, this potential vector is becoming increasingly prevalent. Whilst drifting litter is transported by natural forces, it is considered by the authors that the presence of anthropogenic marine litter is a human influence, without which, fouling species would not be able to make use of prevailing currents to spread rapidly.

Qu. 4.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	likely	**CONFIDENCE**	medium

Response: *Schizoporella japonica* is known to attach to a wide range of substrates (Collin et al 2015). And is known to attach to drifting debris and litter (McCuller & Carlton 2018) which is often found in and around heavily populated areas or sites with heavy use, including marinas and ports. These are also places where colonization of natural and man-made substrates by *S. japonica* is most likely to occur. The species is hermaphroditic and reproduction is likely to take place year-round. Larvae are brooded, with a short larval phase of a few hours before settlement (Ryland et al 2014). It is therefore likely that drifting debris, which comes into close contact with infested areas will be colonized before continuing to drift, potentially to new sites in the RAA. Propagules will be produced and released during the drift and potentially in more concentrated quantities if and when the object becomes stranded or sinks.

Qu. 4.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	likely	**CONFIDENCE**	medium

Response: Based on previous introductions, *S. japonica* has a very high potential to survive transportation attached to drifting debris (McCuller & Carlton 2018). The survivable temperature range is high (-1.4 - 30ºC) (Loxton et al 2017, CABI 2019). Reproduction through asexual budding and colony growth is likely provided conditions are favourable and propagule production (which takes place year round) is also likely. In fact, colonies found on drifting debris in the Pacific have been found bearing live larvae (McCuller & Carlton 2018), demonstrating the potential for sexual reproduction to occur 'in transit'.

Qu. 4.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	moderately likely	**CONFIDENCE**	low

Response: Physical removal of all anthropogenic debris from the shore, may remove fouling colonies, especially if objects are sensibly disposed of at a land-based facility. However, debris removal is currently inadequate in most of the RAA (and certainly in the areas where is *S. japonica* currently found) to effectively remove all potential objects.

Qu. 4.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very likely	**CONFIDENCE**	high

Response: Ancestrula measure 350-400 x ~300 µm (Ryland et al 2014) and at this stage are extremely difficult to detect. A single ancestrula can develop and reproduce asexually to form colonies of hermaphroditic, reproductive individuals. Developed colonies are easier to detect measuring several centimetres across and being bright orange. However, confusions over identification and the large number of taxonomically similar native species reduce the likelihood that the species will be identified and intercepted except by experts.

Qu. 4.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	moderately likely	**CONFIDENCE**	low

Response: Non-feeding ciliated larvae are brooded and released, these larvae persist for only a few hours (Loxton et al2017), but during this time are able to transfer from their host to an adjacent surface. Multiple hard substrates have proved a suitable habitat for settlement and growth, including equipment and structures associated with aquaculture and natural hard substrates, such as shell, rock and boulders (see review in Loxton et al 2017). It would be necessary for host debris to either snag and hold on a floating object with suitable substrate for settlement, or for the drift object to become beached in the proximity of suitable habitat, these are both events which are considered to be moderately likely.

Qu. 4.9d. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	moderately	**CONFIDENCE**	medium

Response: Large quantities of anthropogenic flotsam drifts throughout the RAA and is transported large distances via currents. The potential for such spread has been demonstrated in Helgoland, when the invasive bryozoan *Watersipora subatra* was found attached to rafting seaweed, thought to have travelled 800km from the English Channel (Kuhlenkamp & Kind 2013). Evidence of *S. japonica* surviving far longer journeys across the Pacific Ocean (McCuller & Carlton 2018) suggests distances such as this would be easily achievable. Spread by this pathway would also likely be sporadic, but potentially over long distances. They would potentially have the additional feature of occurring in natural and less expected locations (i.e. harbours or marinas) thus be more likely to go undetected.

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very difficult	**CONFIDENCE**	high

Response: Due to the open nature of the marine environment in which the described pathways of spread occur, complete containment would be extremely difficult, costly and most likely impossible.

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	rapidly	**CONFIDENCE**	medium

Response: Following the introduction of *S. japonica* in the UK in, or not long before 2009, the species has spread widely throughout the UK and into Ireland. The discontinuous nature of the current known range coupled with its presence in locations associated with recreational boating (Loxton et al 2017) suggests that this vector has been used effectively to spread *S. japonica* within the area rapidly, expanding its range by more than 900miles in 10 years. It is not unreasonable to suppose that this rate of spread could continue throughout the RAA. Movements of bivalves are also likely to result in sporadic, discontinuous spread, but will generally be restricted to movement within each member state due to restrictions on the movement of live bivalves between states. However, in the USA, over 40 years the wide range and distribution of *S. japonica* (Alaska to California) has primarily been attributed this vector. Other potential vectors of spread, include rafting on anthropogenic materials and movement with equipment associated with renewable energy and aquaculture, are far more difficult to predict However it is believed that such spread will also take place sporadically and over long distances, resulting in discontinuous populations.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	rapidly	**CONFIDENCE**	low

Response: Under future climate change scenarios, there is no evidence to suggest that any of the impacts summarized in 4.11 will change. However, increased storminess may result in increased levels of anthropogenic flotsam entering the sea, resulting in an increased likelihood of rafting as a pathway.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	moderate	**CONFIDENCE**	medium

Comment: *Schizoporella japonica* is able to colonize newly cleared areas and competitively exclude other species as demonstrated by the colonization of a marina in North Wales following clearance to control another invasive fouling species (Ryland 2014). *S. japonica* is a strong competitor for space, able to inhibit growth of adjacent species of bryozoa and mussels, sometimes causing mortality (Treibergs 2012; Macleod et al 2016). It has been reported that *S. japonica* may be less able to settle on space which is already occupied (Sutherland 1978), which suggests that otherwise healthy and un-impacted natural systems may be less at risk from the deleterious effects of invasion by the species.

It is most commonly associated with marinas and harbours, however it has been observed in the natural environment in Alaska and now in Scottish waters. In Alaska, *S. japonica* has been shown to outcompete native bryozoans (Dick et al 2005). Dick et al (2005) observe that the species remains a dominant feature of such communities, occupying several square metres and occupying large portions of the underside of boulders in sites along the west coast of North America at least 40 years subsequent to its first introduction. This suggests that these impacts may be long lasting and significant.

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	moderate	**CONFIDENCE**	low

Comment: Due to the relatively recent incursion of the species into Europe (Ryland et al 2014), there is little evidence of impacts on biodiversity from the area. In the few sites currently occupied the level of establishment varies from occasional, sparse colonies on man-made structures in two out of three marinas in South-West England (personal observation by author), to well established colonies on man-made and natural substrate in Scotland (Loxton et al 2017).

The authors could find no studies specifically quantifying the impact of *S. japonica* on biodiversity, however its ability to overgrow and outcompete native species (Treibergs 2012; Macleod et al 2016) and to competitively exclude other species, as has been demonstrated during incursions in North Wales (Ryland et al 2014), suggests a potential to impact benthic and under boulder communities intertidally and in the shallow subtidal. In North Wales, such dominance was still apparent more than a year after first arrival.

S. japonica is known to overgrow and smother bivalves, including the mussel *Mytilus edulis* often smothering and causing mortality (Treibergs 2012; Macleod et al 2016). Loxton et al (2017) identified that the biological communities most at risk of impact through competition for space are mussel beds and boulder communities, both of which are UK priority habitats (Macleod et al 2016). Bivalves such as mussels create biologically diverse habitat and loss of this habitat which may be caused by overgrowing by *S. japonica* has the potential to reduce biodiversity, although, again no studies have been conducted to support this theory or quantify the extent to which a reduction in biodiversity may or may not result. The findings of Dick et al (2005) suggest that any impacts may be long lasting (more than 40 years) and significant.

The authors could find no evidence to suggest that hybridization with native species is likely to cause negative impacts on native biodiversity, however with the uncertainties around taxonomy of the genus (Loxton et al 2017), it should not be entirely discounted as a potential impact.

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	moderate	**CONFIDENCE**	low

Comment: The authors could find no studies specifically quantifying the impact of *S. japonica* on biodiversity, however its ability to overgrow and outcompete native species (Treibergs 2012; Macleod et al 2016) and to competitively exclude other species (Ryland et al 2014) suggests a potential to impact benthic and under boulder communities intertidally and in the shallow subtidal. Dick et al (2005) observe that the species remains a dominant feature of such communities, occupying several square metres and occupying large portions of the underside of boulders in sites along the west coast of North America at least 40 years subsequent to its first introduction suggest that these impacts may be long lasting and significant.

S. japonica is known to overgrow and smother bivalves, including the mussel *Mytilus edulis* often smothering and causing mortality (Treibergs 2012; Macleod et al 2016). Bivalves such as mussels create biologically diverse habitat and loss of this habitat which may be caused by overgrowing by *S. japonica* has the potential to reduce biodiversity, although, again no studies have been conducted to support this theory or quantify the extent to which a reduction in biodiversity may or may not result. Studies by Powell et al (1970) observed that whilst *S. japonica* settles and grows on live bivalves, colonies seemed to develop more readily on empty shells, suggesting that impacts on live bivalves may be lower than anticipated.

Although little species-specific information is available, there is evidence that *S. japonica* is able to colonies *Zostera marina* (sea grass) beds (Williams 2007). This is a particularly sensitive and biologically important habitat within the RAA and any potential loss or damage caused by incursions by invasive species could severely impact biodiversity. Similar fouling organisms are known to reduce growth and photosynthesis in sea grass, with heavy infestations leading to canopy collapse and clearance of beds (Williams 2007).

The authors could find no evidence to suggest that hybridization with native species is likely to cause negative impacts on native biodiversity, however with the uncertainties around taxonomy of the genus (Dick et al 2005; Ryland et al 2015), it should not be entirely discounted as a potential impact.

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

Including the following elements:

- Native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- Protected sites impacted, in particular Natura 2000
- Habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats

- The ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	Moderate	**CONFIDENCE**	Low

Comment: The current sites where the species has been identified as present or invasive sit within or in close proximity to a number of sites protected under the habitats directive. At particular risk of impairment are 'Reefs', which include bivalve beds as well as a range of subtidal and intertidal hard substrates, which are all suitable habitat for *S. japonica* to colonize. In these habitats, *S. japonica* has the potential to dominate and alter substrate and to competitively dominate native species (see5.2 & 5.3 for details), potentially reducing the condition of the interest feature.

As a species recognized as having the potential to impact natural systems and species, its presence and spread within member states could lead to a reduced environmental status under the Marine Strategy Framework Directive (EU Directive 2008/56/EC). In the UK, *S. japonica* has been added to a list of species, which will be monitored to ensure GES (Good Environmental Status) for Descriptor 2.

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	Moderate	**CONFIDENCE**	Low

Comment: 'Reefs' as designated for protection under the Habitats Directive include bivalve beds as well as a range of subtidal and intertidal hard substrates which are all suitable habitat for *S. japonica* to colonizes. In these habitats, *S. japonica* has the potential to dominate and alter substrate and to competitively dominate native species (see5.2 & 5.3 for details), potentially reducing the condition of the interest feature.

Is known to overgrow and smother bivalves, including the mussel *Mytilus edulis* often smothering and causing mortality (Treibergs 2012; Macleod et al 2016). Mussel beds are listed as features of interest and protected in several member states, due to their commercial importance, but also because they create biologically diverse habitat, which is often associated with protected birds. Such habitat is, for example essential for the survival of the common eider *Somateria mollissima* which is included in the

European Red List for Birds as ‘vulnerable’. No studies have been conducted to support this theory or quantify the extent to which a loss of habitat may or may not result.

S. japonica is able to colonize *Zostera marina* (sea grass) beds (Williams 2007), which are a feature of conservation importance in many member states and are included as features of ‘Sandbanks, which are slightly covered by sea water all the time’. Similar fouling organisms are known to reduce growth and photosynthesis in sea grass, with heavy infestations leading to canopy collapse and clearance of beds (Williams 2007).

As a species recognized as having the potential to impact natural systems and species, its presence and spread within member states could lead to a reduced environmental status under the Marine Strategy Framework Directive (EU Directive 2008/56/EC).

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?
• For a list of relevant services use the CICES classification V5.1 provided as an annex. • Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being. • Quantitative data should be provided whenever available and references duly reported. • In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	Major	**CONFIDENCE**	Low

Comment: *Provisioning – Biomass – Reared Aquatic Animals/ Provisioning – Biomass –Wild Animals*

No information has been found on this issue, although *S. japonica* is known to overgrow and smother bivalves, including the commercially important mussel *Mytilus edulis* often smothering and causing mortality (Treibergs 2012; Macleod et al 2016). It is also know that *S. japonica* fouls oysters, likely impairing their potential market value, by reducing product quality and increasing the cost associated with preparation and packaging, problems common to a number of fouling organisms (Watson et al 2009).

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	Minimal	**CONFIDENCE**	Medium

Comment:

Provisioning – Biomass – Reared Aquatic Animals/ Provisioning – Biomass –Wild Animals

No information has been found on the issue, although is known to overgrow and smother bivalves, including the mussel *Mytilus edulis* (an important food resource with cultural significance in many parts of the RAA) often smothering and causing mortality (Treibergs 2012, Macleod et al 2016).

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	major	**CONFIDENCE**	Low

Comment:

Provisioning – Biomass – Reared Aquatic Animals/ Provisioning – Biomass –Wild Animals

No information has been found on this issue, although is known to overgrow and smother bivalves, including the commercially important mussel *Mytilus edulis* often smothering and causing mortality (Treibergs 2012; Macleod et al 2016). It is also know that *S. japonica* fouls oysters, likely impairing their potential market value, by reducing product quality and increasing the cost associated with preparation and packaging, problems common to a number of fouling organisms (Watson et al 2009).

Throughout the Celtic Seas, Greater North Sea and northern Bay of Biscay regions, shellfisheries are commercially and culturally important. Mussel and oyster growing in open systems are the most important in terms of output and cultural significance. France (*Mytilus edulis)* and Spain (*Mytilus galloprovincialis*) produce 280 thousand tonnes of mussels per year (FAO 2019), around two thirds of European mussel production. UK and Ireland also produce significant quantities of mussels, often exported to Belgium and the Netherlands where they constitute an important element of traditional cuisine and are culturally important. It is unlikely that infestation of mussel and oyster operations will be destroyed by *S. japonica* alone, however cumulative impacts with other invasive and native fouling organisms may cause widespread problems. Impacts on bivalve health, quality and productivity (as described by Watson et al 2009), may increase costs, and reduce competitiveness with alternative, global providers of bivalve product, resulting in loss of revenue and in the most extreme cases loss of culturally significant activities.

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	Moderate	**CONFIDENCE**	Low

Comment: We could find no evidence of economic loss caused specifically by *S. japonica*. However, associated costs are likely to be similar to those incurred due to other similar fouling organisms and fouling communities with which *S. japonica* might be associated. This would include the culture and harvest of shellfish and the fouling of commercial and recreational vessels. *S. japonica* is found in association with a range of structures and gear associated with commercial activities (Dick et al 2005; Ryland et al 2014; Loxton at al 2017) It is therefore likely that costs will have been incurred by commercial and recreational boat owners and marina operators as a result of having to clean and maintain vessels and equipment more often.

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	Minor	**CONFIDENCE**	Low

Comments: We could find no evidence of economic loss caused specifically by *S. japonica* , anywhere in the EU, however arrival in the RAA is potentially too recent and current range too small for any such costs to have been incurred.

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	Major	**CONFIDENCE**	Low

Comments: We could find no evidence of economic loss caused specifically by *S. japonica*, however associated costs are likely to be similar to those incurred due to other similar fouling organisms and fouling communities with which *S. japonica* might be associatedh. Although not possible to quantify direct costs, it is likely that the main areas affected might be:

Culture and harvest of shellfish: Quality and volume of product likely to be impaired if heavy fouling occurs (Watson et al 2009). It is possible that areas with known populations of *S. japonica* may be closed for export of live mussel seed and other bivalves within the European Union and indeed with each individual state. Restrictions on the movement of mussels from Morecombe Bay (UK) have been imposed for the past year due to the presence of Chinese mitten crabs, impacting the region's largest fishery. Similar measures may be required should *S. japonica* arrive in the area, to prevent further spread.

Fouling of commercial and recreational vessels: S. japonica is found in association with a range of structures and gear associated with commercial activities (Loxton at al 2017). It is therefore likely that costs will be incurred by commercial and recreational boat owners due to: an increased requirement for fuel due to drag; increased maintenance and repair due to fouling of pipes and moving parts; increased cleaning and maintenance of vessels and equipment.

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer "No information has been found on the issue". This is necessary to avoid confusion between "no information found" and "no impact found".

RESPONSE	Major	**CONFIDENCE**	Low

Comments: No information has been found on the issue directly relating to *S. japonica* however, the species is one of many fast growing, encrusting invasive species for which regular hull cleaning and maintenance has become a necessary requirement. This activity varies in cost depending on vessel size and location and between member states.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	Major	**CONFIDENCE**	Low

Comments: No information has been found, however restrictions on movement of vessels and shellfish, which could be implemented (as in the Morecombe Bay, UK mussel seed fishery for *Eriocheir sinensis*) to prevent spread may result in loss of earnings in the fishing industry or for marina owners and commercial shipping companies.

Additional costs may be incurred should *S. japonica* move into areas where shellfishing or shellfish culturing takes place, around the coasts of the UK, Ireland, France, Belgium, Netherlands, Denmark, Spain, Portugal and Germany. Incursion may result in loss or deterioration of product and/ or increased processing time, but should be considered along with costs already associated with removal of native and non-native fouling organisms.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	Moderate	**CONFIDENCE**	low

Comments: The authors could find no evidence to suggest that *S. japonica* might directly impact human health. The ability for the species to grow on ropes and equipment associated with maritime industries and recreational boating (Loxton et al 2017) may make gear heavier and increase the risk of back injury or falling in when trying to retrieve lines, ropes and other gear. However, there is no reason to suppose that such an impact would be any worse that caused by other fouling organisms (native and non-native). *S. japonica* may in fact competitively exclude larger organisms such as solitary and colonial ascidians and macro algae, which would otherwise increase the mass of rope fouling.

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	Minimal	**CONFIDENCE**	Low

Comments: No additional impacts could be found.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	Minor	**CONFIDENCE**	Low

Comments: No additional impacts could be found for *S. japonica* specifically, however it is possible that the provision of additional hard substrate of calcareous biogenic structures created by *S. japonica* might provide additional settlement space and refuge for additional non-native species on what might otherwise be an inhospitable or homogenous substrate. The congeneric species *S. errata* is known to have had such an effect on mudflats in San Francisco Bay (Zabin et al 2010). Studies showed that 74% of the species of known origin colonizing the new ‘bryolyths’ were non-native.

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	Moderate	**CONFIDENCE**	Low

Comments: No additional impacts could be identified, but this does not mean that none are possible. Alteration of habitat, indirect impacts on predators and wider ecosystem impacts may occur.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	Moderate	**CONFIDENCE**	Low

Comments: No information could be found about impacts of predators, parasites or pathogens on the successful establishment and impacts of *S. japonica*. Predation on newly settled propagules by grazing mulluscs, flatworms and other invertebrates is likely, although the extent to which this will impair the establishment and subsequent impact of the species is not known. Based on the spread and current distribution of the species on the west coast of the USA (Dick et al 2005), where similar predator and pathogen assemblages can be found, it is not considered likely that this will be an important factor inhibiting impacts caused.

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	Major	**CONFIDENCE**	Medium

Comments: Although very little information exists, which can be used to quantify past or current impacts of *S. japonica*, a number of potential impacts have been considered based on best judgment and supported by published observations and lessons learned from similar and in particular congeneric species.

The ability of *S. japonica* to overgrow and outcompete native species (Treibergs 2012; Macleod et al 2016) and to competitively exclude other species (Ryland et al 2014), suggests a potential to impact benthic and under boulder communities intertidally and in the shallow subtidal. Such impacts may be long lasting. *S. japonica* is also known to overgrow and smother bivalves, including the mussel *Mytilus edulis* often smothering and causing mortality (Treibergs 2012; Macleod et al 2016). Bivalves such as mussels create biologically diverse habitat and loss of this habitat, which may be caused by overgrowing by *S. japonica*, has the potential to reduce biodiversity.

The potential to overgrow bivalves has additional potential impacts, including economic and social/ cultural and is likely to impede the functioning of ecosystem services as described in 5.10. Any such impacts are likely to be long lasting, but should be considered alongside the impacts of other fouling organisms.

The ability of *S. japonica* to colonies a range of man-made structures, suggests that costs will be incurred in order to keep gear clean and in working order, and to maintain the efficiency of vessels. Again, the costs of these activities should be considered alongside the existing cost of antifouling and hull and equipment cleaning as is likely to replace existing fouling species, some of which are larger and bulkier and likely to generate more drag and additional weight.

It is possible that *S. japonica*, as with *S. errata* (Zabin et al 2010), will create new habitat, suitable for settlement by new non-native species and this might result in indirect impacts resulting from incursions of other non-native, invasive species.

Although little species-specific information is available, there is evidence that *S. japonica* is able to colonies *Zostera marina* (sea grass) beds (Williams 2007). This is a particularly sensitive and biologically important habitat within the RAA and any potential loss or damage caused by incursions by invasive species could severely impact biodiversity. Similar fouling organisms are known to reduce growth and photosynthesis in sea grass, with heavy infestations leading to canopy collapse and clearance of beds (Williams 2007).

The areas within the RAA most likely to be impacted are considered to be the Celtic Seas, Greater North Sea and the Bay of Biscay, with the northern Iberian coast above Portugal also likely to be impacted. Due to the primarily northern distribution and the apparently more rapid colonization by the species in colder conditions (Loxton et al 2017), it is considered that growth and spread may be faster in the northern parts of the UK, Scandinavia and Ireland than along the coast of France, Spain, Belgium, Netherlands and Germany. Resulting in higher levels of impact in these areas.

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	Major	**CONFIDENCE**	Low

Comments: Under future climate change scenarios as discussed previously, there is no evidence to suggest that any of the impacts summarized in 5.19 will change.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Introduction*	Very likely	High	It is considered that, given the proximity of known populations to the RAA ([n particular Norway) and presence of established populations within the RAA (UK) it is very likely that *S. japonica* will be introduced unintentionally to new member states within the RAA. Populations exist in densities and locations that make attachment to vessel hulls, equipment, debris, and commercially harvested and transported bivalves very likely. It is considered by the authors that there are currently insufficient measures in place to control fouling organisms, on recreational vessels to reduce this potential source of introduction. Additionally, there is a constant flow of vessels between the sites currently hosting the species and uninfected sites within the RAA.
Summarise Entry*	Very likely *(Northern)* Moderately likely *(Black Sea)* Unlikely *(Southern Iberian coast, Mediterranean)*	High *(Northern)* Medium *(Mediterranean and Black Sea)*	Due to the sessile nature of *S. japonica* entry would mainly be due to release of propagules from reproductively viable introduced or passing populations. Reproduction is by the release of relatively large, brooded ciliated larvae, which settle and metamorphose within hours of release. They are capable of selecting optimal attachment locations and once settled, a single propagule is able to reproduce asexually, forming an extensive colony of hermaphroditic zooids, with a high fecundity. Production and release of propagules is constant and continues throughout the year, increasing the chance of spread from sources of introduction. Propagules, newly settled individuals and small colonies are difficult, if not impossible to detect without very close and detailed inspection and even large, conspicuous colonies may be overlooked due to difficulties with identification and recognition. Provided conditions are suitable for reproduction to occur and colonies to survive, it is therefore considered that there is a very high likelihood of entry into the RAA. These conditions exist in the entire Celtic Seas, Greater North Sea regions, Bay of Biscay and the Iberian Coast north of Bilbao, Spain. Conditions are considered unsuitable due to salinity levels falling outside the known tolerable range of the species on the Iberian coast south of Bilbao, and the Mediterranean (too high) and the Baltic (too low). Future predicted salinity decreases in the Iberian coast region might make this area habitable in the next 50 years. Whilst

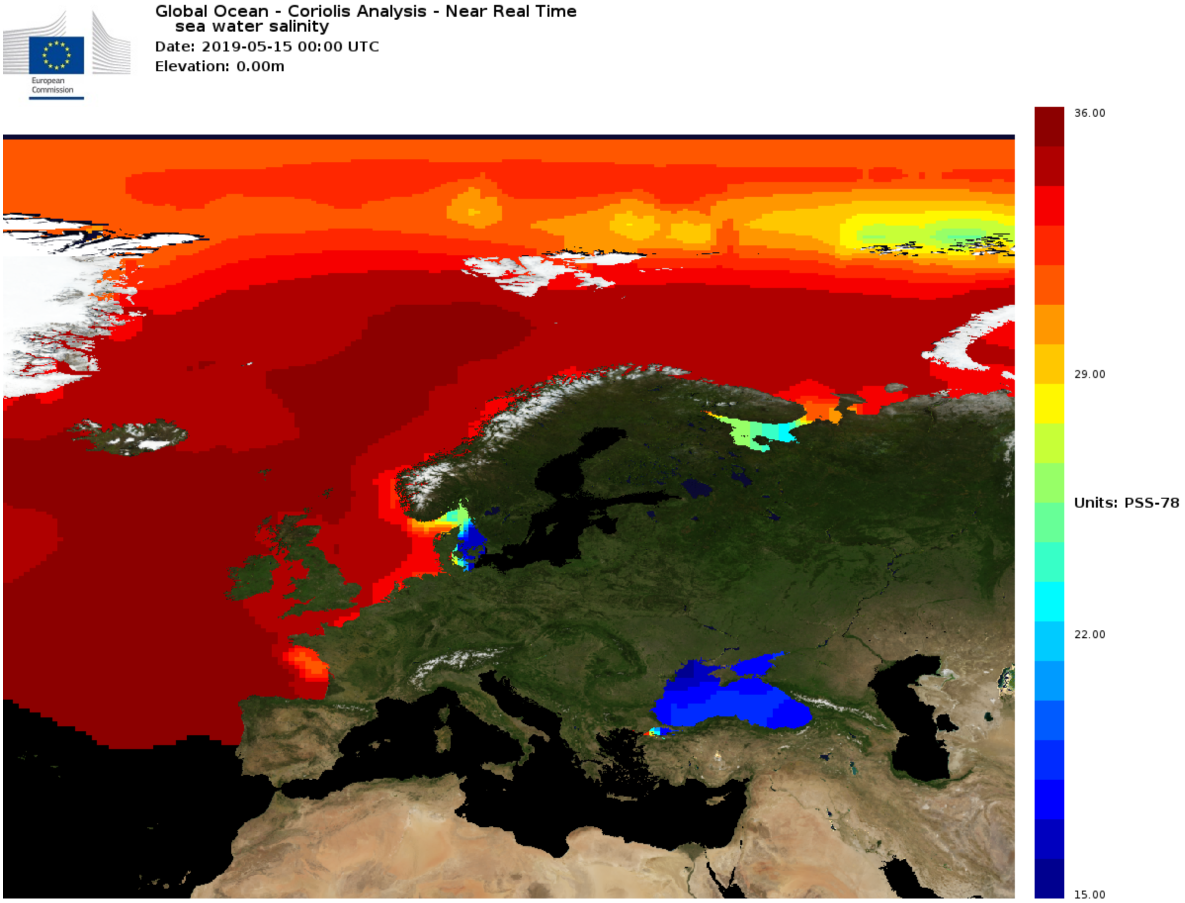
			salinity in the Black Sea may be suitable, it is unclear whether passage through the Mediterranean would result in reproductively viable colonies, therefore unless transported by another route, entry may be impaired.
Summarise Establishment*	Very likely	High	*S. japonica* has demonstrated the ability to grown fast and spread rapidly under a range of environmental conditions. It is capable of establishing large, long-lasting populations with low genetic diversity and a single successfully settling propagule is capable of growing into a large, reproducing colony. It is capable of overgrowing and competitively excluding existing fouling communities (possibly due to its ability to reproduce constantly and throughout the year) and to rapidly colonize newly available substrate, excluding competing organisms. Available habitat is ubiquitous throughout the RAA, with *S. japonica* capable of settling and growing on a range of natural and man-made substrates. Establishment is considered unlikely on the Iberian coast south of Bilbao, and in the Mediterranean (salinity too high) and the Baltic (salinity too low).
Summarise Spread*	Rapidly	Medium	Due to the short-lived nature of propagules and limited dispersal range, natural, self initiated spread is likely to be slow from introduced populations, however local, dense populations are likely. *S. japonica* has however demonstrated traits, which allow it to spread effectively by other means. Spread by transport with vessels, is likely to be over long distances, but resulting in discontinuous populations, but with regular vessel movements between member states and the life history traits discussed spread is likely to be rapid. Movement of bivalves is very likely to facilitate spread within member states, including between sea regions (e.g. Atlantic to Mediterranean oyster transplantations) due to limited regulation regarding the internal transfer of bivalves. Bivalve movement may also result in spread between states, although this will vary due to restrictions on bivalve movements in some states. Some natural spread by rafting of natural objects (seaweed), but perhaps more so anthropogenic flotsam may result in long-range, sporadic, disjointed introductions throughout the RAA.
Summarise Impact*	moderate	Medium	*S. japonica* is able to overgrow and outcompete native species and continue to dominate systems for in excess of 40 years. Few studies have been undertaken to quantify impacts of this species,

			however information about similar species and impacts of dominating encrusting invasive species suggests a likelihood that this will result in loss of biodiversity and habitat alteration. An ability to overgrow and cause mortality of bivalves has implications for the environmental status of protected biogenic 'reefs', but also loss of food provision in the form of bivalve culture and resulting economic impacts. Bivalve fisheries and aquaculture (particularly mussels and oysters) have important economic and cultural significance through out the potentially affected areas of the RAA. Economic impacts on these activities may result from increased processing time, reduced product quality and quantity and ultimately loss of customers in a globally competitive market. Impacts on recreational and commercial shipping operations include increased cleaning and maintenance costs and higher fuel costs due to fouling. The ability of the species to colonize a range of man-made equipment, gear and vessels implies potential costs for the aquaculture industry, fishing industry and renewable marine energy companies. Costs may be incurred as a result of increased maintenance requirements, damage caused by increased drag and weight of gear or health issues caused by lifting and moving heavily fouled gear. However, these costs should be considered alongside the cost of dealing with existing fouling communities native and non-native (including some larger, faster growing organisms), which occurs throughout the RAA and which may be competitively excluded by *S. japonica.*
Conclusion of the risk assessment (overall risk)	High	Medium	Based on current conditions within the RAA, The score applies to: The Celtic Seas, Greater North Sea, Bay of Biscay and Iberian Coast north of Bilbao Spain. It is considered that conditions within these areas are suitable for the establishment of *S. japonica* where habitat is suitable and the life history traits make association with potential vectors of introduction and spread very likely. Populations capable of seeding new populations are already present in the RAA (UK) and in Norway, which is in close proximity to the RAA and shares many transport links suitable for transporting the species. Once arrived, the ability of the species to grow fast from a limited propagule bank and competitively exclude native species makes it likely that impacts on the environment and economic interests will occur and that these will be widespread or locally severe. Limited

			information exists about the specific impacts of the species and the taxonomic uncertainty regarding the species means that some information may be difficult to locate and possibly unreliable. This means that some of the scores and predictions may require revision, as more is understood about the nature of the species in the future. It is however considered by the authors that information about the species alongside information about similar species and fouling communities makes it possible to make the predictions here with some confidence.

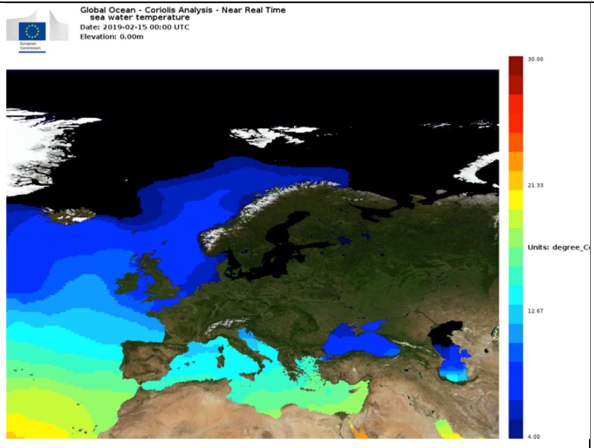
*In current climate conditions and in foreseeable future climate conditions

Appendix 1: Climatic variables maps

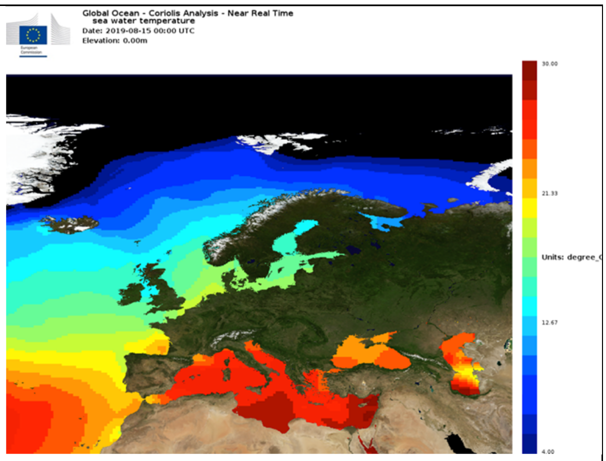


June 2019 Salinity analysis data from Global Ocean- Real time in-situ observations objective analysis. Black areas signify regions outside the known tolerable salinity levels for *S. japonica*.
Future Predicted changes: Baltic likely to reduce by 50-80% due to ice melt (EEA 2017) from http://marine.copernicus.eu

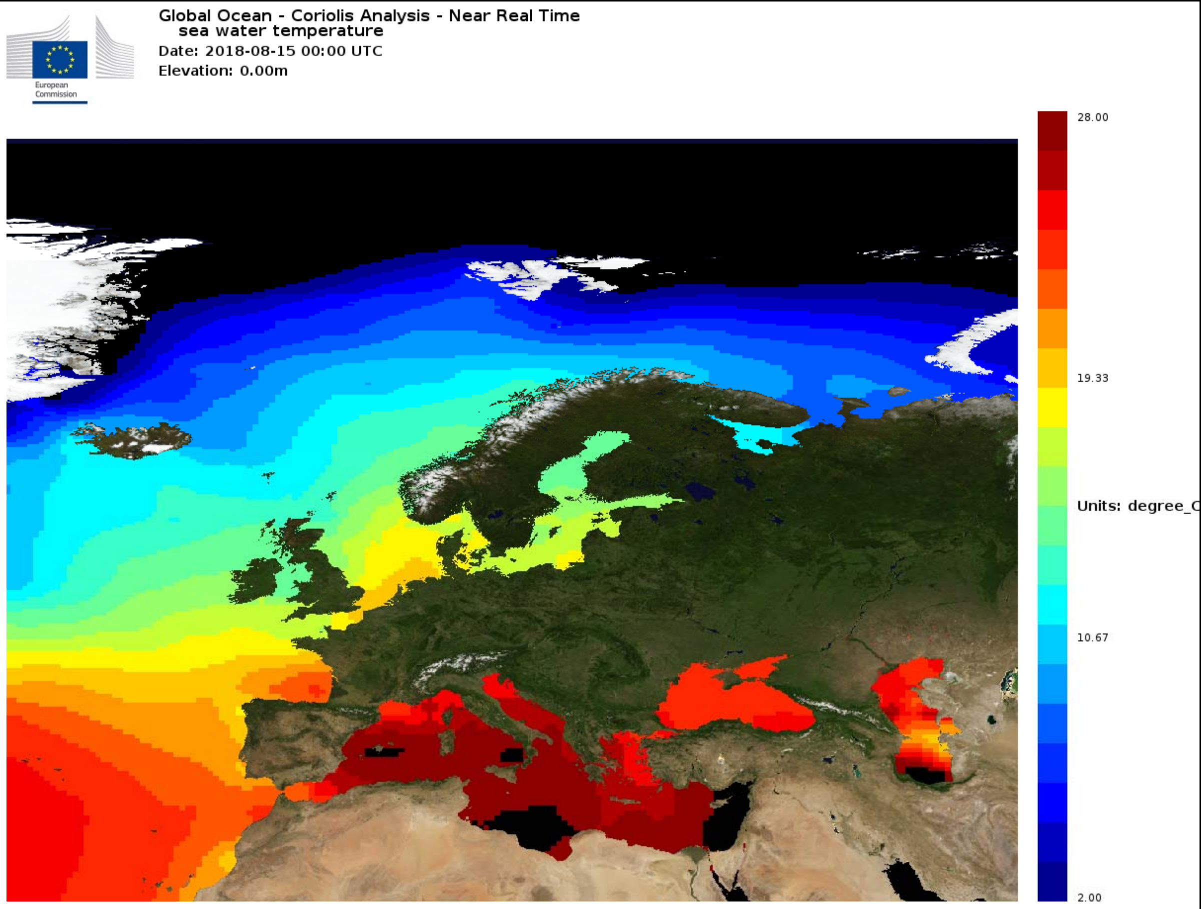
Sea Temperature Maps From: http://marine.copernicus.eu/services-portfolio/access-to-products/?option=com_csw&view=details&product_id=INSITU_GLO_TS_OA_NRT_OBSERVATIONS_013_002_a



Average Sea Temperature: Feb 2019 – Min – max range known survival tolerances. Black areas considered outside minimum or maximum survivable temperature range.



Average Sea Temperature: Aug 2019 – Min – max range known survival tolerances. Black areas considered outside minimum or maximum survivable temperature range.



Average Sea Temperature: Aug 2019 – Min – max range known survival tolerances reduced by 2.0°C to represent RCP 4.5 possible increase by 2065 Black areas considered outside minimum or maximum survivable temperature range.

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Zabin, C. J., Obernolte, R., Mackie, J. A., Gentry, J., Harris, L., & Geller, J. (2010). A non-native bryozoan creates novel substrate on the mudflats in San Francisco Bay. Marine Ecology Progress Series, 412, 129-139. doi:10.3354/meps08664

Distribution Summary

Please answer as follows:

Yes	if recorded, established or invasive
–	if not recorded, established or invasive
?	Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.
For data on marine species at the Member State level, delete Member States that have no marine borders.
In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	-	-	-	-	-
Belgium	-	-	yes	yes	-
Bulgaria	-	-	yes	yes	-
Croatia	-	-	?	-	-
Cyprus	-	-	-	-	-
Czech Republic	-	-	-	-	-
Denmark	-	-	yes	yes	-
Estonia	-	-	-	-	-
Finland	-	-	-	-	-
France	-	-	yes	yes	-
Germany	-	-	yes	yes	-
Greece	-	-	-	-	-
Hungary	-	-	-	-	-
Ireland	yes	yes	yes	yes	yes
Italy	-	-	?	-	-
Latvia	-	-	-	-	-
Lithuania	-	-	-	-	-
Luxembourg	-	-	-	-	-
Malta	-	-	-	-	-
Netherlands	-	-	yes	yes	-
Poland	-	-	-	-	-
Portugal	-	-	?	yes	-
Romania	-	-	yes	yes	-
Slovakia	-	-	-	-	-
Slovenia	-	-	-	-	-
Spain	-	-	yes	yes	-
Sweden	-	-	yes	yes	-
United Kingdom	yes	yes	yes	yes	yes

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	-	-	-
Atlantic	yes	yes	yes	yes	yes
Black Sea	-	-	yes	yes	-
Boreal	-	-	-	-	-
Continental	-	-	-	-	-
Mediterranean	-	-	?	-	-
Pannonian	-	-	-	-	-
Steppic	-	-	-	-	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea	-	-	-	-	-
Black Sea	-	-	yes	yes	-
North-east Atlantic Ocean	yes	yes	yes	yes	yes
Bay of Biscay and the Iberian Coast	-	-	Yes	Yes	-
Celtic Sea	yes	yes	yes	yes	yes
Greater North Sea	yes	yes	yes	yes	yes
Mediterranean Sea	-	-	-	-	-
Adriatic Sea	-	-	?	-	-
Aegean-Levantine Sea	-	-	-	-	-
Ionian Sea and the Central Mediterranean Sea	-	-	-	-	-
Western Mediterranean Sea	-	-	-	-	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Description**	**Frequency**
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact, and other impacts**
	Question 5.1-5	*Question 5.6-8*	*Question 5.9-13*	*Question 5.14-18*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[6]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

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[6] Not to be confused with “no impact”.

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	**Description**
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence *and/or* Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area *and/or* Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous *and/or* The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred *and/or* Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty *and/or* The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) *and* Impacts are recorded at a comparable scale *and/or* There are reliable/good quality data sources on impacts of the taxa *and* The interpretation of data/information is straightforward *and/or* Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES "classes")
Provisioning	**Biomass**	**Cultivated *terrestrial* plants**	Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes; Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a source of energy *Example: negative impacts of non-native organisms to crops, orchards, timber etc.*
		Cultivated *aquatic* plants	Plants cultivated by in- situ aquaculture grown for nutritional purposes; Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an energy source. *Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.*
		Reared animals	Animals reared for nutritional purposes; Fibres and other materials from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide energy (including mechanical) *Example: negative impacts of non-native organisms to livestock*
		Reared *aquatic* animals	Animals reared by in-situ aquaculture for nutritional purposes; Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an energy source *Example: negative impacts of non-native organisms to fish farming*
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for nutrition; Fibres and other materials from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a source of energy *Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)*
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for nutritional purposes; Fibres and other materials from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a source of energy *Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)*

	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[7]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection *Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.*
		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection)

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[7] Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that are resonant in terms of culture or heritage; Characteristics of living systems that enable aesthetic experiences *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance*
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	**Spiritual, symbolic** and other interactions with natural environment	Elements of living systems that have symbolic meaning; Elements of living systems that have sacred or religious meaning; Elements of living systems used for entertainment or representation *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning*

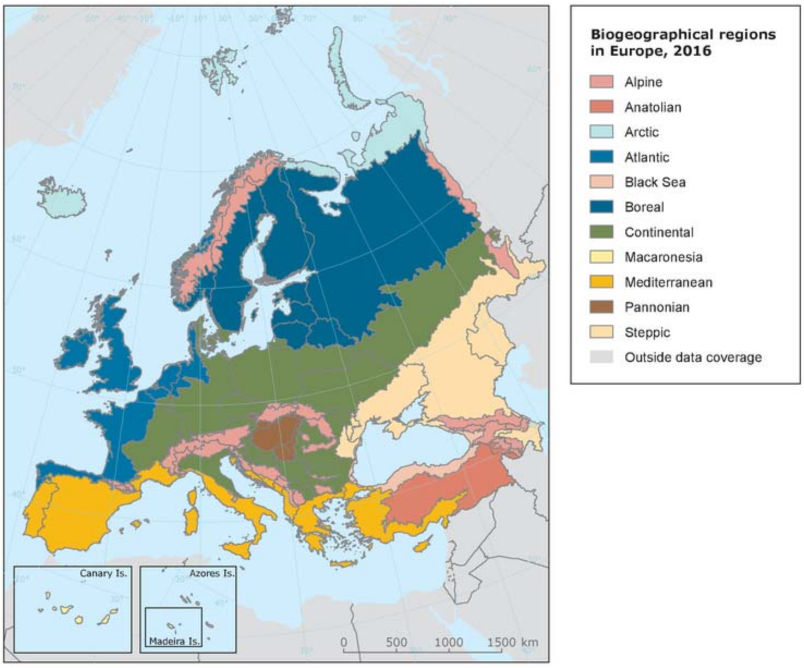
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

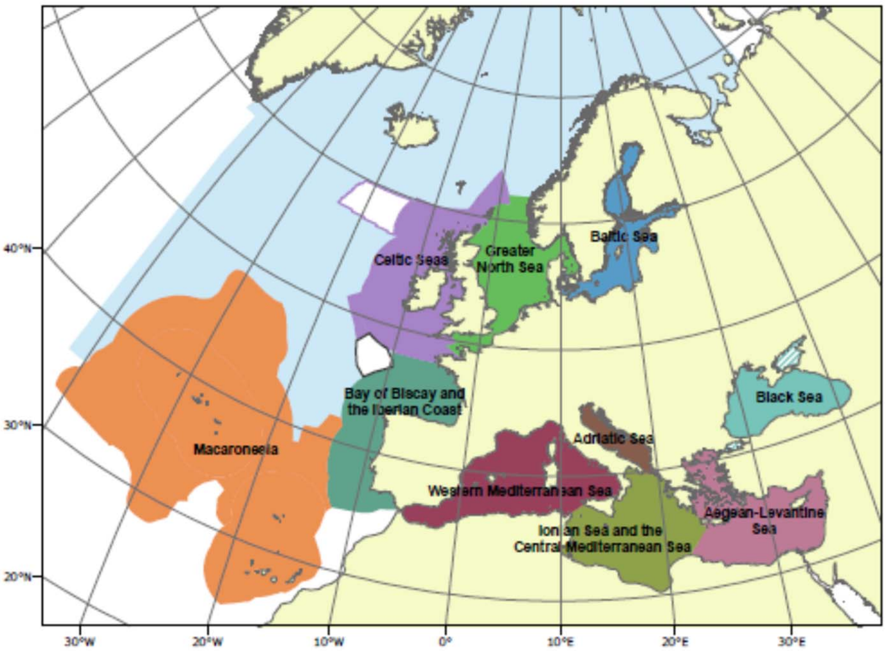
ANNEX V EU Biogeographic Regions and MSFD Subregions

See https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2 , http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf





ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968

Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Schizoporella japonica***
Species (common name)	**Orange ripple bryozoan**
Author(s)	**Jack Sewell**
Date Completed	**14/10/2019**
Reviewer	**Marika Galanidi**

Summary [1]

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Schizoporella japonica is a species of Bryozoan, native to the Western Pacific. It forms encrusting colonies on a range of man-made and natural substrates. It is considered a fouling pest with potential to smother or outcompete native species. It is invasive on the Eastern Pacific coast of North America and non-native, established populations are present in Norwegian harbours as well as Malaysia. Historic misidentification and taxonomic confusion mean that distribution elsewhere is not certain, however populations are thought to be invasive in Australia.

Within the risk assessment area (RAA, the territory of the European Union, excluding the outermost regions), currently established populations occur only around the UK including the coast of Scotland, Scottish Northern Isles, North Wales and Plymouth, Devon. It has been recorded in Ireland.

Prevention of introduction:

Hull fouling movements: Hull fouling is controlled via anti-fouling paints and cleaning practices both in the commercial and recreational sectors. In contrast to ballast water, there are currently no specific conventions or legally binding international frameworks to control biofouling. In 2011, the IMO[a] adopted Resolution MEPC.207(62) outlining the Guidelines for the Control and Management of Ships' Biofouling to Minimize the Transfer of Invasive Aquatic Species. The Guidelines are supplemented by the Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft circulated as MEPC.1/ Circ.792. While in some cases these guidelines will be followed and the risk from well-maintained vessels will be relatively low, those operators that do not follow the guidelines will present a much higher risk.

Contaminant on Aquaculture:

Council Regulation (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed to minimise the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. According to the regulation, all aquaculture operators who intend to introduce an alien species or translocate a locally absent species must first apply for a permit from the competent authority of the Member State where the transfer will take place. The potential for newly introduced stock to introduce a harmful invasive species such as *S. japonica* would warrant a reason to decline application to transfer to the area. Although the rules regarding introduction of bivalves into the RAA is robust, exceptions for the Pacific oyster *Magallana gigas* (listed as *Crassostrea gigas* in Annex IV of R. 708/2007) and the potential for non-compliance and illegal introductions means that the measures currently in place may not provide total protection.

Eradication and management

Many of the measures described depend on the early identification of populations in source locations. The cryptic nature of *S. japonica*, taxonomic uncertainty and the level of expertise required to confirm identification of the species make early identification and interception extremely unlikely. This is exacerbated by the small size of founder colonies and of the propagules. The use in future of eDNA monitoring may increase the chances of early detection. The open nature of marine systems mean that once established, eradication would not be possible and management of pathways would prove problematic and likely impractical. However, management of impacts over limited areas is possible, for example, manual cleaning of fouled bivalve stock and hull cleaning, however any such activities would need to be repeated regularly and would likely incur high (potentially excessive) financial costs.

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[a] IMO: International Maritime Organization

Detailed assessment			
	Description of measures[2]	**Assessment of implementation cost and cost-effectiveness (per measure)[3]**	**Level of confidence[4]**
Methods to achieve prevention[5]	**Managing the pathway "Ships' Hull Fouling"** **(Commercial and Recreational)** Hull fouling is controlled via anti-fouling paints and cleaning practices both in the commercial and recreational sectors. In contrast to ballast water, there are currently no specific conventions or legally binding international frameworks to control biofouling. In 2011, the IMO adopted Resolution MEPC.207(62) outlining the Guidelines for the Control and Management of Ships' Biofouling to Minimize the Transfer of Invasive Aquatic Species. Recreational crafts shorter than 24 m in length may instead find relevant guidance in IMO's 2012 document "Guidance for Minimizing the Transfer of Invasive Aquatic Species as	While in some cases these guidelines will be followed and the risk from well-maintained vessels will be relatively low, those operators that do not follow the guidelines will present a much higher risk. Therefore, the organism is considered likely to be able to survive passage. Anti-fouling paints have limited service life and require re-application at regular intervals, they seem to be efficient for up to 1-1.5 years – thereafter heavy fouling can start occurring (Sylvester et al., 2011; Frey et al., 2014). Whilst antifoulants slow down or reduce settlement, there are numerous parts of a typical ship's hull prone to colonisation by fouling organisms (dry dock strips, propellers and rudders, unpainted areas, nooks and crevices, areas of damage). These areas require physical cleaning to remove fouling (Zabin et al 2016). The Bryozoan *Watersipora subatra* is known to be tolerant of copper-based antifouling paint -a feature thought to have contributing to its success as a invasive species - The settlement of *W. subatra* can provide substrate suitable for the settlement of other species (Floerl et al 2004). The authors were unable to find information about the ability of *S. japonica* to resist antifouling treatments, however it should be noted that in the congener *S. errata,* Cu (copper) based antifouling coatings on boat hulls can prevent growth of *S. errata* and stop its spread to	**Medium**

	Biofouling (Hull fouling) for Recreational Craft" (MEPC, 2012). The two main practices for the removal of biofouling from ships' hull are: • Dry docks • In-water cleaning (IWC), there has been a proliferation of new IWC technology in the past decade (e.g. https://www.ecosubsea.com/, http://econetsaustralia.com/) that capture debris and render it non-viable through e.g., UV treatment (for a review see Zabin et al., 2016). It should be noted that certain regional regulations regarding biofouling management have already entered into force, e.g. in Australia, New Zealand, parts of the USA and the Galapagos Marine Reserve (Zabin et al., 2018).	new locations (Piola and Johnston 2006). It is likely, given the species' ability to settle on a range of man-made and natural substrates, that it would be one of the species likely to benefit from the provision of new settlement space provided by tolerant early settlers like *W. subatra.* Vessel cleaning during dry-docking in a shipyard generates a very low biosecurity risk because the debris is sent to local deposit and residue water from cleaning is collected (Bohn et al., 2016). Maintenance during dry-docking also involves the re-application of anti-fouling paint. Vessels with larger docking intervals (up to 5 years) increasingly choose intermediate cleaning of the hull with in-water technologies (Bohn et al., 2016). In-water cleaning used as an additional tool to dry-docking; can be combined with loading/unloading activities, is faster and can cost as little as 1/5 the cost of dry docking (Hagan et al., 2014). However, in-water cleaning of hulls, especially without capturing the biofouling debris, might represent a higher risk of introducing NIS[b] relative to land-based cleaning in dry-docks with land based waste disposal since physical disturbance of the fouling communities may trigger the release of propagules or viable gametes (Hopkins & Forrest, 2008). Dislodged individuals are likely to settle on surrounding benthos and if surrounding habitat is suitable, may spread and become established. Large fragments (>600mm^2) of fouling turf in particular are likely to survive	

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[b] NIS: non-indigenous species, term used in the Marine Strategy Framework Directive, synonym of "alien species" as used in the framework of Regulation (EU) 1143/2014

		and in many cases reattach to surrounding suitable substrate, more so than smaller fragments (up to $18mm^2$) although this is very much dependent on local conditions (Zabin et al 2016). New technologies are currently being developed and trialled globally, which aim to remove and sterilise hull fouling using specialist equipment. Such initiatives may provide a relatively safe, cost effective alternative to dry-docking and the value of such systems in reducing the spread of invasive species should be given careful attention. See for example: http://franmarine.com.au/projects/envirocart/ The cost will be borne by the shipping companies or private vessel owners (for fishing and recreational vessels). It is likely that stakeholders will be generally supportive of any requirements to maintain hull cleanliness due to the widely understood economic and safety benefits of vessel maintenance. Costs associated with hull fouling management measures are not specific to *S. japonica* but refer to all sessile marine NIS. Traditional dry-docking costs hundreds of thousands of euros, and the cost of reapplying a new layer of antifouling amounts to half the total cost. Indicatively, typical in-water cleaning of a 180-200 m container vessel conducted by companies in the US east coast would take approximately two days for an entire hull; cost of application in the range of €17-43k.	

		Emerging IWC systems are currently available in only a few locations worldwide, and are more costly than traditional methods (Zabin et al., 2016). The cost to marina owners of establishing a biosecure treatment facility for the disposal of hull fouling material was estimated to be at least £45-50K in the UK (DEFRA, 2012).	
	Managing the pathway "Contaminant on Aquaculture" Current EU legislation in the form of the Alien Species in Aquaculture Regulations (708/2007) (EC 2007) prevents the deliberate introduction of non-native species for aquaculture, unless potential risks are mitigated within Member States. Any proposals to introduce a new species from outside the region would require a detailed assessment of potential risks and proof that measures were in place to ensure no environmental impact from the new introductions. This would include detailed assessment of potential secondary introductions. .	It should be noted that *Schizoporella japonica* has been recorded as a fouling species on mussels (Loxton et al 2017) and Pacific oysters (Dick et al 2005). However, the Pacific oyster *(Magallana gigas)* is listed in Annex IV of Council Regulation 708/2007 as an exception and can be moved without any risk assessment or quarantine within the RAA. Also the regulation does not apply to movements of locally absent species within the Member States "except for cases where, on the basis of scientific advice, there are grounds for foreseeing environmental threats due to the translocation, Art. 2 para. 2. It is likely that a known incursion by an invasive species like *Schizoporella japonica* would constitute an environmental threat and thus activate this exception. While the legislation presents a robust road-block to the potential introduction of the species from within or outside the RAA, any legislation is only as good as its enforcement. Maintaining high levels of enforcement in relation to these regulations is therefore essential. Additional complications over the movement of *M. gigas* specifically might lead to areas of legal uncertainty, which may also reduce the effectiveness of the legislation.	**Medium**

	The ICES Code of Practice on the Introductions and Transfers of Marine Organisms (ICES, 2005) recommends the procedure for introduced or transferred species which are part of current commercial practice. The procedure states clearly that: a) all products should originate from sources in areas that meet current codes, such as the OIE International Aquatic Animal Health Code or equivalent EU directives. b) if required, there should be inspection, disinfection, quarantine or destruction of the introduced organisms and transfer material (e.g., transport water, packing material, and containers) based on OIE or EU directives. Lastly, using hatchery-produced seed reduces the risk of introduction and spread through stock/seed transfers, provided facilities are, themselves uncontaminated.	As this legislation is already implemented there would be no additional costs associated specifically with management for this species. Additional regulations and transposed law regarding damaging activities in Natura 2000 sites may also provide a legislative barrier to activities likely to impair the condition of interest features and thus prohibit activities likely to result in the introduction of invasive species. Restrictions on transfers based on the risk associated with the source areas is an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk NIS are available. The cost of sourcing, obtaining the necessary permission and harvesting or transferring alternative bivalve (seed) populations will vary with production and current/future harvesting location and practice and may be considerable for the producer – this mainly applies to countries with less strict intra-state shellfish transport regulations. Non-compliance with regulations may be reduced with more effective policing/monitoring and stronger sanctions but would have a high associated cost. Many of the measures described depend on the identification of populations in source locations. There are many difficulties with identification of *S. japonica* in the field, taxonomic uncertainty, and detection issues caused by the small size of founder colonies and propagules. There is therefore a high chance that populations may go undetected	

		during inspections, giving the illusion that source cultures of bivalves are 'clean' and pose no risk, when in fact they do. The use of eDNA monitoring could make this more feasible.	
Methods to achieve eradication[6]	Theoretically, eradication may be possible for localised, newly established populations at low densities with limited dispersal capabilities or no local recruitment, using mechanical or chemical treatments where all fouling (native and non-native) is removed/killed. However, this would require an early warning system, monitoring efforts and a removal programme. Including the support of specialist taxonomic experts.	The small size and cryptic nature of the early life stages of *S. japonica* would make initial interception very difficult. The encrusting and brittle nature of the species means it is very unlikely that scraping it off substrates would remove all parts of a colony which us likely to fragment into small pieces. Use of chemicals such as bleach to kill all fouling in an enclosed site, may be feasible, but is unlikely to result in complete eradication; when this method was trialled in Holyhead marina, Wales to remove the Carpet sea squirt *Didemnum vexillum*, it was *S. japonica* which subsequently settled comprehensively over the cleared areas of pontoons. It is more likely that an adoption of proactive 'good practice' with regards to general fouling removal would prove most effective – in other words, assuming presence in fouling communities unless proved otherwise. Once colonies are visible and easily detectable they will likely already be reproductively viable, and eradication would be unlikely. In a review of eradication attempts in the marine environment, Locke and Hanson (2007) suggest that eradication of any marine invasive species is not possible once established. Additionally, local eradication would require ongoing, long-term, regular interventions due to the ongoing risk of spread from well-established, surrounding populations. Due to the	**High** Eradication campaigns in the wild have not been attempted anywhere in the invaded range. Eradication attempts have been made for similar marine species in the past with no or little long-term success.

		year-round reproduction exhibited by the species, any such activities would need to be regular and continuous. Without a clear action plan in place and regular monitoring, the taxonomic uncertainties around the species and identification difficulties would very likely delay any eradication activity as has been observed in previous eradication attempts for marine species (Locke & Hanson 2007). Resulting in the closure of the 'window' of time available between arrival and establishment. Cost of unsuccessful attempted eradication of D. vexillum in Holyhead marina - £385,000 in first 3 years. Ongoing monitoring after 3 years estimated to be £20,000 per year.	
	E1. Early warning systems / awareness raising The species requires specialized taxonomic expertise for its identification, such that awareness raising and early warning systems are better designed with a focus on the training of professionals who are likely to encounter/collect it during the course of monitoring or other marine survey activities, as well as potentially affected stakeholders (i.e. shellfish growers, those undertaking cleaning of vessel hulls and	A guide to the identification of early settlement stages in IAS present in fouling communities has been produced (Bishop et al 2015) to support the early detection of the species. However, given the small size of propagules and early colonies, the likelihood of monitoring detection attempts failing is very high. The introduction of eDNA monitoring could increase the likelihood of early detection. An effective platform for such knowledge exchange can be the network INVASIVESNET, which aims to facilitate greater understanding and improved management of invasive alien species (IAS) and biological invasions globally by linking new and existing networks of interested stakeholders (Lucy *et al.*, 2016). Work with stakeholders, likely to encounter the species, for example vessel owners and those engaged in the	**Medium**

	scientists monitoring fouling communities on man-made structures).	aquaculture industry to train and raise awareness could result in an effective early warning system that would help identify and remove colonies during the early stages of invasion. However, It would likely be more effective to raise awareness of the need for good practice in terms of hulls fouling management generally in order to engender a culture of good biosecurity.	
	Monitoring and early detection Monitoring of potential high risk areas of introduction, by regular visual inspections or by use of molecular tools and DNA barcoding. Such activity would require specialist training and knowledge.	While there are several legislative requirements for monitoring of biodiversity in the marine environment (e.g. Water Framework Directive, Habitats Directive), the Marine Strategy Framework Directive is the only one that explicitly requires the monitoring of marine non-native species. At this point in time monitoring effort and methods vary considerably between Member States, and therefore their ability to detect new introduction. However, co-ordination of monitoring through the Regional Seas Conventions (e.g. HELCOM and OSPAR) is helping to increase the regional effectiveness of monitoring. The European Union Regulation (No 1143/2014) on the prevention and management of the introduction and spread of invasive alien species (IAS) requires early detection of new introductions of listed species, but currently there is only one marine species listed (*Eriocheir sinensis*). In addition to statutory monitoring efforts effective engagement with those undertaking mariculture and fishing activities, in addition to researchers working in or in close proximity to sites of potential introduction could provide value in establishing an effective early warning system. Such activities would have an initially high financial cost, but systems such as those deployed in Great Britain (GBNNSIP Alert System) might ultimately provide part of an effective monitoring system. Difficulties with identifying *S. japonica* (Ryland et al 2014) - in particular similarities to native	**Medium**

		species - mean that monitoring by trained individuals might be necessary and effective identification materials in multiple languages would be required in order to effectively prepare any would-be participants. Novel methods of detection such as the use of eDNA are starting to be developed for a range of non-native species (Bean et al 2017). While such methods are currently in development, these could aid considerably in the detection of new introductions. As these processes are already in place there would be no additional costs associated specifically with this species.	
	Removal methods	Although no accounts of removal of *S. japonica* specifically could be found, general methods of antifouling may be effective on populations fouling man-made objects, in particular vessel hulls. As previously described, hull cleaning during dry-docking is a viable method of physical removing fouling populations from vessels and the least likely to result in accidental release into the wild. In order to prevent further spread, the careful management of spoil is necessary. Cost of removal and cleaning of recreational vessels increases with vessel size and can vary greatly depending on available facilities, ranging from hundreds to thousands of NZ$ per day (Inglis et al 2011). For larger vessels up to 5,000 tonnes, may exceed NZ$37,000. Once out of the water, fouling can be removed by scraping or jet washing A number of in-water removal systems are now available Inglis et al (2011) provides a comprehensive review of options available and approximate costs. The cost of cleaning a 50m vessel are thought to range from NZ$16,000 using a water blast robot system (taking 1 day) to NZ$3,900	

		using an encapsulation process (2-14days). Heat treatment and brush systems are also suggested. For a 200m vessel, costs range from NZ$127,100 for water-blast robot system (3 days) to NZ$30,000 using the encapsulation method (4-14 days). The encapsulation method involves covering the vessel or gear with an impervious material and utilizing chemical treatment to kill fouling species. With all in-water methods, there remains a risk of contaminating surrounding area with propagules and dislodged organisms. Financial costs are likely to be associated with delays to movement and operation. Additionally, once introduced, treatment of other infected structures, such as marina pontoons would be required in order to avoid repeated introductions and further spread.	
Methods to achieve management[7]	**Managing the impacts of established populations**	There are no accounts of effective management of invasive populations of *S. japonica,* once established in the wild. Once present, management would be extremely difficult. Reactive management for example physical cleaning of fouled mariculture stock may be effective in the short term, but would require regular interventions in order to maintain clean stock should local source populations be present. Intervention options are limited due to the need to retain quality and condition of stock, which precludes many high-impact measures, including chemical treatment and mechanical treatment. Cleaning by hand is the most likely method, which can be very costly and time consuming. In heavily impacted areas, regular cleaning may be required in order to prevent reduced growth and impaired product	**low**

		quality. Hypothetically, such an ongoing cost may make culturing effected species uneconomical.	

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach with be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

- **High**: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.

- **Medium**: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low**: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion. This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Describe the methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This section should assume that the Member State is currently free of this species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Describe the methods that might be applied by Member States to support eradication: i.e. complete removal, including rapid response or eradication of the species. This section should assume that the species has been found within the Member State and consider the options for eradication, accepting that this may or may not be possible. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

7 Describe the methods that might be applied by Member States to support population control. i.e. reducing spread, protecting assets, limiting impacts, containment, localised rapid responses or long-term control. This section should assume that the species is now sufficiently well established within the Member State that eradication is no longer a reasonable prospect. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention (contract No 07.0202/2018/788519/ETU/ENV.D2).

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