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

Contract No 07.0202/2017/763379/ETU/ENV.D2

Final Report

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Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention
Contract No 07.0202/2017/763379/ETU/ENV.D2

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Abstract: There is a need to provide evidence-based assessments of the risks posed by invasive alien species (IAS) to underpin policies and prioritise action. Here we present ten risk assessments and associated management annexes for IAS selected following a prioritisation exercise. The selected species were *Polygonum polystachyum*, syn. *Koenigia polystachya* (Himalayan Knotweed), *Solenopsis richteri* (Black Imported Fire Ant), *Solenopsis geminata* (Tropical fire ant), *Cydalima perspectalis* (box tree moth), *Callosciurus finlaysonii* (Finlayson's squirrel), *Xenopus laevis* (African clawed frog), *Fundulus heteroclitus* (mummichog), *Morone americana* (white perch), *Perna viridis* (Asian Green mussel), *Lagocephalus sceleratus* (Silver-cheeked toadfish). 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: Pour établir des priorités d'action pour les espèces exotiques envahissantes (EEE), il est urgent de développer des évaluations de risque qui soient fondées sur des éléments probants. Nous présentons ici dix évaluations des risques et des annexes relatives à la gestion pour chacune des espèces sélectionnées à la suite d'un exercice d'établissement de priorité. Les espèces sélectionnées étaient *Polygonum polystachyum*, syn. *Koenigia polystachya* (renouée à nombreux épis), *Solenopsis richteri* et *S. geminata* (fourmi de feu), *Cydalima perspectalis* (pyrale du buis), *Callosciurus finlaysonii* (écureuil de Finlayson), *Xenopus laevis* (xénope lisse), *Fundulus* *heteroclitus* (choquemort), *Morone americana* (bar blanc d'Amerique), *Perna viridis* (moule verte Asiatique) et *Lagocephalus sceleratus* (poisson-ballon). Les évaluations des risques et des annexes relatives à la gestion ont été produits parallèlement à des tâches visant à élaborer et 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 le rapport coût-efficacité. Les évaluations des risques serviront de preuves pour déterminer si les espèces cibles devraient être considérées comme des espèces exotiques envahissantes préoccupantes pour l'Union selon le Règlement (UE) 1143/2014 relatif à la prévention et à la gestion de l'introduction et de la propagation des espèces exotiques envahissantes.

Samenvatting: Het beleid en beheer rond invasieve uitheemse soorten (IUS) dient onderbouwd te worden met risicoanalyses die gebaseerd zijn op de best beschikbare kennis. Dit rapport presenteert tien risicoanalyses voor soorten die vooraf via een prioriteringsoefening geselecteerd werden. Het gaat om *Polygonum polystachyum*, syn. *Koenigia polystachya* (Afgaanse duizendknoop), de vuurmieren *Solenopsis richteri* en *S. geminata*, *Cydalima perspectalis* (buxusmot), *Callosciurus finlaysonii* (Finlayson's eekhoorn), Xenopus laevis (Afrikaanse klauwkikker), *Fundulus heteroclitus* (mummichog, killivis), *Morone americana* (Amerikaanse zeebaars), *Perna viridis* (groene Aziatische mossel) en de kogelvis *Lagocephalus sceleratus*. 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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There are 10 annexes to this report:

Annex 1: Risk assessment & annex on measures for *Polygonum polystachyum*, syn. *Koenigia polystachya* (Himalayan Knotweed)
Annex 2: Risk assessment & annex on measures for *Solenopsis richteri* (Black Imported Fire Ant)
Annex 3: Risk assessment & annex on measures for *Solenopsis geminata (Tropical fire ant)*
Annex 4: Risk assessment & annex on measures for *Cydalima perspectalis (box tree moth)*
Annex 5: Risk assessment & annex on measures for *Callosciurus finlaysonii (Finlayson's squirrel)*
Annex 6: Risk assessment & annex on measures for *Xenopus laevis* (African clawed frog)
Annex 7: Risk assessment & annex on measures for *Fundulus heteroclitus* (mummichog)
Annex 8: Risk assessment & annex on measures for *Morone americana* (white perch)
Annex 9: Risk assessment & annex on measures for *Perna viridis* (Asian Green mussel)
Annex 10: Risk assessment & annex on measures for *Lagocephalus sceleratus* (Silver-cheeked toadfish)

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Oli Pescott	Co-Lead of Task 3, quality control, providing expertise on terrestrial and freshwater plant species
Dan Chapman	Co-Lead of task and Lead implementation of model projections of invasive distributions for all risk assessments
Jodey Peyton	Facilitate meetings, workshops, and provide project support
Karsten Schönrogge	Co-lead of Task 2, project supervision, quality control
Steph Rorke	Coordination of data across project
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Wolfgang Rabitsch	Co-lead Tasks 1 and 5, Support to all tasks
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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 first renewal of the Study Contract No 070202/2016/740982/ETU/ENV.D.2 "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 1 to reflect decisions taken during the final workshop of 13 October 2017, and ensure consistency with the provisions of the Delegated Act (Commission 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). The updated template, approved with the European Commission (DG Environment), was used for completing all 10 risk assessments under task 3.

Task 2: The list of species to be assessed was developed in collaboration with the European Commission. At the kick-off meeting of 23 November 2017 the European Commission was presented with a draft list of 23 priority species, with indications for the species of highest priority for Risk Assessments according to the views of the project team. 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). The Commission provided comments on this list and in agreement with experts from within the project team, 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. However, one species was included that is currently widespread, *Cydalima perspectalis*. The ten selected species were:

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

1. *Polygonum polystachyum* (Himalayan Knotweed)
2. *Solenopsis richteri* (black Imported Fire Ant)
3. *Solenopsis geminata* (tropical fire ant)
4. *Cydalima perspectalis* (box tree moth)
5. *Callosciurus finlaysonii* (Finlayson's squirrel)
6. *Xenopus laevis* (African clawed frog)
7. *Fundulus heteroclitus* (mummichog)
8. *Morone americana* (white perch)
9. *Perna viridis* (Asian Green mussel)
10. *Lagocephalus sceleratus* (silver-cheeked toadfish)

Task 3: The risk assessments were developed over the entire duration of the contract and involved experts from within the project team, along with 15 additional experts (7 acting as lead authors, 7 as peer-reviewers and one as both lead author and peer-reviewer). 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 11-12 October 2018, in which each risk assessment was presented, discussed and amended to ensure overall comprehensiveness and consistency in approaches by assessors.

Task 4: The template for management was subject to minor modifications at the start of the year 2 study, on the basis of discussions held during the final workshop of year 1 of 13 October 2017, and the kick-off meeting of year 2 of 23 November 2017. 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 11-12 October 2018, with the only exception of including a description of the options for disposal of the plants and animals removed from the environment.

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 exécutoire

Pour établir des priorités d'action pour les espèces exotiques envahissantes (EEE), il est urgent de développer des évaluations de risque qui soient fondées sur des éléments probants. Les évaluations de risque sous-tendent les politiques relatives aux EEE de plusieurs façons: informer la législation; justifier les restrictions relatives aux activités commerciales ou de consommation; prioriser la surveillance et la réponse rapide. Les évaluations des risques réalisées dans le cadre de la présente étude fournissent des éléments probants permettant de déterminer si des espèces cibles doivent être envisagées pour inclusion dans la liste d'EEE préoccupantes pour l'Union du Règlement EU 1143/2014 sur la prévention et la gestion de l'introduction et de la propagation des espèces exotiques envahissantes (règlement EEE).

Nous présentons ici les résultats d'une étude articulée autour de quatre tâches:

Tâche 1: Mettre à jour et adapter sur base de l'expérience acquise le modèle d'évaluation des risques
Tâche 2: Développer la liste des espèces à évaluer
Tâche 3: Préparer les évaluations de risque
Tâche 4: Recueillir des éléments probants sur les techniques de gestion, les coûts de mise en œuvre et le rapport coût-efficacité

Tâche 1: Le modèle d'évaluation des risques a été modifié par rapport à la version utilisée la première année pour refléter les décisions prises lors de l'atelier final du 13 octobre 2017 et pour garantir la cohérence avec les dispositions de l'acte délégué (règlement d'exécution (UE) 2018/968 de la Commission du 30 décembre 2017) du 30 April 2018 complétant le règlement (UE) n° 1143/2014 du Parlement européen et du Conseil en ce qui concerne l'évaluation des risques liés aux espèces exotiques envahissantes). Le modèle mis à jour, approuvé avec la Commission européenne (DG Environnement), a été utilisé pour compléter les 10 évaluations de risque de la tâche 3.

Tâche 2: La liste des espèces à évaluer a été élaborée en collaboration avec la Commission Européenne à la suite d'un exercice d'établissement de priorité. Lors de la réunion de lancement du 23 novembre 2017, une liste de 23 espèces prioritaires a été présenté à la Commission européenne, avec des indications sur les espèces prioritaires pour les évaluations de risques, conformément aux points de vue de l'équipe de projet. L'équipe du projet a été divisée en cinq groupes thématiques d'experts: les animaux d'eau douce, les espèces marines, les plantes (y compris d'eau douce), les invertébrés terrestres et les vertébrés. Les groupes ont été invités à sélectionner les EEE identifiée comme étant de priorité élevée ou très élevée pour la réalisation d'analyses de risque lors d'un précédent exercice d'analyse prospective (rapport final du contrat ENV.B.2/ETU/2014/0016) ainsi que toute nouvelle espèce jugée émergente et en consultant la liste développée par Carboneras et al (2017). La Commission a formulé des observations sur cette liste et, en accord avec les experts de l'équipe du projet, 10 espèces ont été sélectionnées comme priorité pour l'évaluation des risques dans le cadre de la cette étude. L'accent a été mis sur les EEE qui ne sont pas encore présents dans l'Union européenne (ou ont une distribution limitée) et qui pourraient avoir un impact négatif sur la biodiversité. Cydalima perspectalis, une espèce largement répandue, a toutefois été incluse. Les dix espèces sélectionnées sont les suivantes:

1. *Polygonum polystachyum* (renouée à nombreux épis)
2. *Solenopsis richteri* (fourmi de feu)
3. *Solenopsis geminata* (fourmi de feu)
4. *Cydalima perspectalis* (pyrale du buis)

5. *Callosciurus finlaysonii* (écureuil de Finlayson)
6. *Xenopus laevis* (xénope lisse)
7. *Fundulus heteroclitus* (choquemort)
8. *Morone americana* (bar blanc d'Amerique)
9. *Perna viridis* (moule verte Asiatique)
10. *Lagocephalus sceleratus* (poisson-ballon)

Tâche 3: Les évaluations des risques ont été élaborées sur toute la durée du contrat et impliquaient des experts de l'équipe de projet mais aussi 15 experts supplémentaires (7 en tant qu'auteurs principaux, 7 en tant que réviseurs et un en tant qu'auteur principal et réviseur). De plus, pour une sélection d'espèces, des modèles de distribution spécifiques ont été développés pour augmenter la base de connaissances requise afin de renforcer le résultat des évaluations de risque. Toutes les évaluations de risque ont fait l'objet d'un examen par au moins deux experts indépendants. Les commentaires des évaluateurs et les réponses respectives des auteurs sont documentés dans ce rapport final d'étude. Les évaluations des risques ont été achevées dans le cadre d'un atelier final de deux jours, organisé du 11 au 12 octobre 2018, dans lequel chaque évaluation des risques était présentée, discutée et modifiée afin de garantir la cohérence des approches des évaluateurs.

Tâche 4: Le modèle pour les annexes relative à la gestion a fait l'objet de modifications mineures au début de l'étude de la deuxième année sur la base des discussions tenues lors du dernier atelier de la première année du 13 octobre 2017 et de la réunion de lancement de la deuxième année du 23 novembre 2017. Des annexes relative à la gestion ont été produites pour chacune des espèces sélectionnées par les équipes produisant les évaluations des risques mais aussi en s'appuyant sur l'expertise de spécialistes supplémentaires. Ces annexes se basent sur les preuves scientifiques clés disponibles à ce jour dans diverses sources avec pour but d'informer les décisions relatives à la gestion du risque.

En conclusion, l'approche d'évaluation de risque utilisée est considérée comme complète et robuste. Aucun changement majeur n'a été suggéré lors de l'atelier final des 11 et 12 octobre 2018, à la seule exception de l'inclusion d'une description des options d'élimination des plantes et des animaux retirés de l'environnement.

Le travail est présenté et documenté dans le rapport final d'étude et comprend un certain nombre de points nécessitant plus de considération ainsi que des recommandations clés. Le rapport s'accompagne de 10 annexes, une pour chacune des dix espèces sélectionnées, ainsi que les annexes de gestion comprenant des informations sur la gestion et les coûts.

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: Marianne Kettunen (IEEP), Wolfgang Rabitsch (EAA), Riccardo Scalera (ISSG)

Other contributors: Etienne Branquart (EPPO), Dan Chapman (CEH), 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 species2 (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.

Although the template including explanatory text was helpful for answering the relevant questions in more detail, it did not completely solve some confusion or inconsistencies in the experts' answers. Naturally, some variation, but also redundancies and duplications exist in the answers and comments of the experts, partly due to the overlap in the questions. Only rarely were answers completely misplaced or unrelated to the question and therefore the template should be seen as a very useful tool for providing comprehensible assessments.

The template was further modified, as agreed by the EC and project team for the second year (contract 07.0202/2017/763379/ETU/ENV.D2), and adapted to meet all requirements specified in the draft of the forthcoming Delegated Regulation supplementing Regulation 1143/2014.

Specifically, the following changes were made:

- The header, title (of the template) and disclaimer were modified.

[2] 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

- The distribution Summary Table was moved to the end of the template, but will be moved back to the front after finalization of the assessments. The "invasive future" column in the Distribution Summary was deleted.
- "General instructions" were added to ensure a better understanding of the purpose of the exercise, e.g. on the redundancy and conciseness of the answers and on the quality of data sources.
- The former EU Chapeau was deleted and the questions incorporated into Section A of the template.
- The "Comment" column in Section A was deleted and more instructions added, e.g. including reference lists with the instruction "[delete as appropriate]" to guarantee completeness.
- All questions were critically reviewed to consider whether there was any redundancy or overlap. As a consequence several questions were deleted or changed (see also changes discussed and approved during the final workshop in the description of task 3). This should also reduce workload for assessors.
- The wording was critically checked and clarified as appropriate. Special attention was given to the incoherent use of "EU/Europe/Union", and most often this was replaced by "the risk assessment area" as above.
- The "Additional Questions on Climate Change" were deleted and the questions incorporated into previous questions throughout the template.
- As already suggested in the first year of the project, the "Very high" score was deleted from the Scoring of Confidence Levels in Annex III.
- Annex IV on Ecosystem Services was updated according to CICES-Version 5.1. Links to Annex V was updated.
- An acknowledgement that this scheme has been developed from the GBNNRA has been added as a footnote: 'This template is based on the Great Britain Non-Native Species Risk Assessment scheme (GBNNRA).'

The project team were invited to seek clarification as required and provide further comments for consideration at the workshop. A number of problems, highlighted over the second year covered by this report, still deserve some consideration for possible improvements. For example:

Level of confidence, categories of impact, pathways categorisation, template structure, species distribution models (SDMs). Details on adjustments that should be considered are provided in the section "Issues and key recommendations" below.

The current risk assessment template is provided on page 37.

Task 2 Develop the list of species to be assessed

Leading experts: Helen Roy (CEH), Karsten Schönrogge (CEH) and Alan Stewart (University of Sussex)

Other contributors: Oli Pescott (CEH), 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 Adriaens (RINF), Sonia Vanderhoeven (Belgian Biodiversity Platform), Olaf Booy (NNSS), Niall Moore (NNSS), 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.

At the kick off meeting in November 2017 the Commission was presented with a draft list of 23 priority species, with indications for the species of highest priority for Risk Assessments according to the views of the project team. The Commission provided comments on this list and in the context of this feedback and discussion with experts from within the project team, the 10 species presented here were agreed as priority for risk assessment in the framework this study. Here we present the process towards this list with justification for the 10 selected species.

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

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 follows:

1. Expert groups were provided with a spreadsheet listing the 95 IAS of EU concern derived by an earlier horizon scanning exercise (see final report of ENV.B.2/ETU/2014/0016) minus the species considered during the previous round of assessments. Accompanying spreadsheets provided separate listings of the species within each taxonomic/ecological group. From these lists, experts were asked to review the list through detailed consideration of candidate species for risk assessment and agree on up to five species. Thus, the expectation was that this process would generate a list of 25 species across all groups from which the Task 2 leaders, in iterative consultation with expert group leaders, would select ten species to go forward to risk assessment in Task 3.
2. Expert groups were reminded that the focus of this exercise is primarily on IAS that are likely to have an impact on biodiversity, but that it is important to record if any of the species might also have potential socio-economic and/or human health impacts (a column in the spreadsheet was provided to record such cases, with brief explanatory notes requested for the general comments column). Groups were also reminded that the focus is on IAS likely to have impacts within the next ten years that either have not yet arrived in the EU or have populations limited to some regions such that there is still scope for action to prevent their further spread within the EU.
3. Initially, expert groups were asked to check whether any of the scores given in the previous horizon scanning study for likelihood of arrival, establishment, spread and impact needed to be revised in the light of more recent information or developments. Groups were asked to suggest revised scores where

appropriate with accompanying justification. Scores were recorded on a 1-5 scale (sometimes fine-tuned to one decimal place), with an overall score calculated as the product of the four individual likelihood scores (maximum = 625).

4. Expert groups were then asked to add any new species that had appeared 'on the horizon' since the previous horizon scanning exercise, especially highlighting any species which they considered should definitely be risk assessed in the near future. In such cases, scores and other standard information was requested for direct comparison with other species already on the list. A list of relevant databases was provided to assist in the search for new species to be added to the list.
5. Experts were asked to provide a clear justification for any changes in scores or the addition of new species in a 'comments on changes to scores' column. Additional references were to be provided in the general comments column where appropriate.
6. Experts were asked to review or add to the secondary information on the potential management of IAS. They were reminded that this set of criteria was secondary to the more important primary scores, but that it could become useful in later stages of ranking the species. Binary 'yes/no' responses were requested for most of the questions, recognising that answers at this stage could be only indicative, given that detailed prescriptions for management would be a major undertaking (to be picked up in Task 4). Rough estimates of management costs were also requested, but again recognising that these could be only very provisional at this early stage.
7. Groups were asked not to consider species that are known to be (i) undergoing risk assessment currently, (ii) already earmarked for risk assessment in the near future, or (iii) covered by other legislation such as that pertaining to plant or animal health. Such species were to be excluded from further consideration for the list of ten priority species. For example, the 16 plant species being covered by the LIFE project IAP-RISK (http://www.iap-risk.eu/) were excluded for this reason.
8. Selection of the top five species in each group was based primarily on the overall scores, but considering and documenting other relevant criteria where appropriate. Group leaders were asked to register any significant differences of opinion between experts about scores for particular species within their group, attempting to resolve these issues by email, Skype etc. and providing information in the general comments column if they had not been resolved.
9. Since developing the list of ten species for risk assessment in study contract no. 07.0202/2016/740982/ETU/ENV.D2, a prioritised list of 900 IAS for consideration for risk assessment has been published (Carboneras et al. 2017). The expert groups were instructed to refer to this manuscript to give consideration to species not included within the EU Horizon Scanning (Roy et al. 2015; Roy et al. in press) but also to note other publications that might be relevant (Nentwig *et al.* 2018; Tanner *et al.* 2017).
10. Finally, experts were asked to provide a concise justification for each of the five selected species including supporting evidence.

Once the group lists had been agreed, Task 2 co-leaders combined them into a single list of 23 species (Table 1) from which the project team proposed to select the ten priority species for risk assessment in Task 3. This list was presented to the Commission for comments at the Kick-Off meeting in November 2017.

Table 1: List of 23 species identified by the expert subgroups for consideration by the project team and European Commission for selecting ten species for risk assessment in 2018 (see Figure 1; Table 2).

Rank from EU Horizon Scanning (Roy et al. 2015)	Expert subgroup	Species	Common Name
HIGH	Plants	*Acacia dealbata*	Silver wattle
HIGH	Plants	*Acer rufinerve*	Grey snake-bark maple
HIGH	Plants	*Miscanthus sinensis*	Chinese silver grass
MEDIUM	Plants	*Pinus patula*	Mexican weeping pine
MEDIUM	Plants	*Polygonum polystachyum*	Himalayan knotweed
HIGH	Terrestrial invertebrates	*Solenopsis richteri*	Black Imported Fire Ant
HIGH	Terrestrial invertebrates	*Solenopsis geminata*	Tropical fire ant
HIGH	Terrestrial invertebrates	*Pheidole megacephala*	Big-headed Ant
	Terrestrial invertebrates	*Wasmannia auropunctata*	Little fire ant
HIGH	Vertebrates	*Axis axis*	Chital
VERY HIGH	Vertebrates	*Pycnonotus cafer*	Red-vented bulbul
VERY HIGH	Vertebrates	*Callosciurus finlaysonii*	Finlayson's squirrel
MORE_WIDESPREAD	Vertebrates	*Xenopus laevis*	African clawed frog
VERY HIGH	Freshwater	*Channa argus*	Northern snakehead
HIGH	Freshwater	*Fundulus heteroclitus*	mummichog
VERY HIGH	Freshwater	*Oreochromis (3 spp.)*	Tilapia
HIGH	Freshwater	*Morone americana*	white perch
HIGH	Freshwater	*Misgurnus anguillicaudatus*	Oriental weatherfish
	Marine	*Callinectes sapidus*	Blue swimming crab
HIGH	Marine	*Perna viridis*	Asian Green mussel
	Marine	*Gracillaria vermiculophylla*	Worm wart weed
	Marine	*Lagocephalus sceleratus*	Silver-cheeked toadfish
	Marine	*Paraleucilla magna*	A calcarean sponge

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 consultation with the 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 (Figure 1; Table 2) is the result of a consensus between all group co-leaders and discussions with the European Commission.

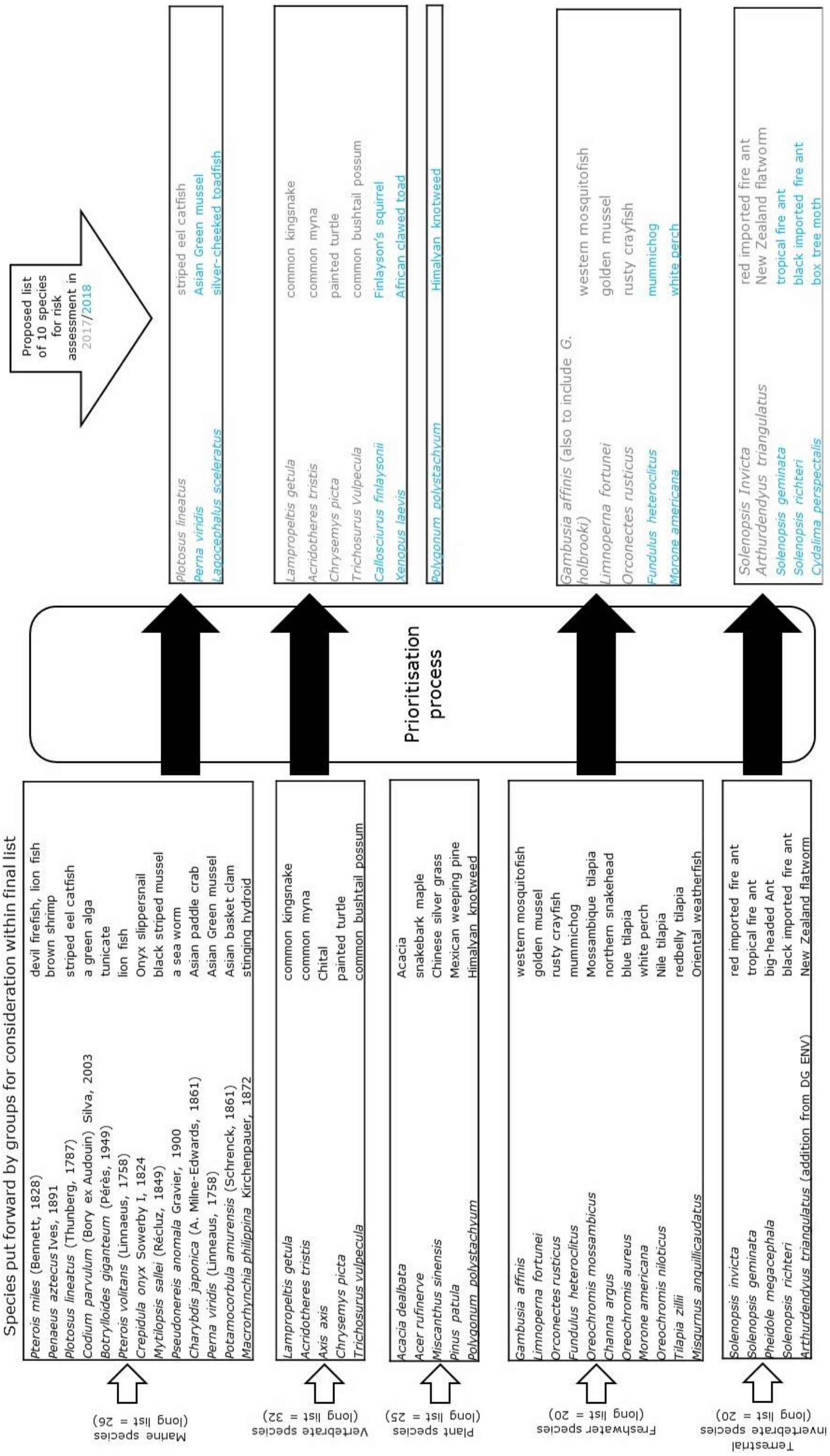

Figure 1. Process of selection of species for risk assessment including updates for 2017/2018. *Cydalima perspectalis* was added on request from the European Commission. The risk assessment for *C. finlaysonii* developed by the Bern Convention secretariat will be reviewed and updated.

Table 2: Species selected for risk assessment highlighting agreement in ranks agreed by EU Horizon Scanning (Roy et al. 2015; Roy et al. in press) and Carboneras et al. (2017)

Expert Group	Species	Common name	EU Horizon scanning rank	Carboneras et al (2017) rank
Terrestrial invertebrates	*Solenopsis richteri*	Black Imported Fire Ant	High	Major
Terrestrial invertebrates	*Solenopsis geminata*	Tropical fire ant	High	Major
Terrestrial invertebrates	*Cydalima perspectalis*	Box tree moth	Not listed	Not listed
Plants	*Polygonum polystachyum*	Himalayan Knotweed	Not listed	Not listed
Vertebrates	*Callosciurus finlaysonii*	Finlayson's squirrel	Very high	Massive
Vertebrates	*Xenopus laevis*	African clawed frog	Not listed*	Major
Freshwater	*Fundulus heteroclitus*	Mummichog	High	Major
Freshwater	*Morone americana*	White perch	High	Major
Marine	*Perna viridis*	Asian Green mussel	High	Major
Marine	*Lagocephalus sceleratus*	Silver-cheeked toadfish	Not listed	Major

*Species considered and discussed through the horizon scanning but not included in the final ranked list because it was already known to be widespread in the EU.

Additional notes to support the list in Table 2:

Solenopsis richteri	Black imported fire ant	Native to South America, but has been introduced to North America, where it first became an important economic and environmental pest. Later, it was partly displaced by *S. invicta*, with which it can hybridize. It is apparently more cold-hardy than *S. invicta*.
Solenopsis geminata	Tropical fire ant	Of South American origin but has a greater worldwide distribution than *S. invicta*. It has also been more often intercepted in Europe than *S. invicta*. Where introduced, it has often become one of the dominant pest ant species, affecting fauna and flora. It may be less cold-hardy than *S. invicta*.
Cydalima perspectalis	Box tree moth	Native to eastern Asia. It was first recorded in Europe in Germany in 2006 and has spread into several other countries since then, threatening the survival of natural box tree stands and, indirectly, a whole cohort of organisms strictly associated with this tree. Added on request by the European Commission.
Polygonum polystachyum	Himalayan knotweed	*P. polystachum* was introduced through gardening trade and effects native communities through

		establishment of dense and tall thickets where it escapes and establishes. Potential for post-invasion spread from fragments of rhizome through human activity and via waterways (similar to Japanese knotweed, *Fallopia japonica*) is high.
Callosciurus finlaysonii	Finlayson's squirrel	Native to South East Asia but introduced to Italy, Singapore and Japan. In Italy it has been linked to severe damage of both deciduous and coniferous trees as a result of bark-stripping behaviour, which can in severe cases kill trees. A risk assessment is available but needs to be checked for completeness.
Xenopus laevis	African clawed frog	Model experimental amphibian used in laboratories pan-globally. Escapees have formed viable and invasive populations in many climates, where individuals are generalist aquatic carnivores, predating on invertebrates, amphibians and fish. It is one of the main known vectors of the fungal pathogen *Batrachochytrium denrobatidis* (which however is already fully widespread in Europe). Management can have some negative side effects, e.g. in cases where poison is used to remove animals.
Fundulus heteroclitus	Mummichog	A fish, native to North America, and introduced in Philippines and Hawaii according to Fishbase. Present in the Iberian peninsula along coastal and estuarine areas. Review available on the impacts on native species and ecosystems through competition and predation (Leunda 2010).
Morone americana	White perch	A fish, native to North America, reported in the Great Lakes; potential impacts on other fish (predation on fish eggs). Absent from EU but a hybrid of *Morone* species, the so-called palmetto bass (*Morone saxatilis* x *M. chrysops*), is known to be imported into the EU, and at least one record in the wild (in Croatia) has been reported.
Perna viridis	Asian green mussel	Affects community structure and trophic interactions. Harbours shellfish toxin. Biodiversity impacts: competitor for space, displacing native species; documented keystone effects on ecosystems (through habitat alteration). Implicated in the loss of oyster beds in the USA. Impact on infrastructure includes fouling, blocking pipes and aquaculture equipment.
Lagocephalus sceleratus	Silver-cheeked toadfish	Possible severe biodiversity impacts through predation and competition; potential to poison naive predators, including seals, seabirds and elasmobranchs.

Discussion of some issues arising

The following points were raised through study contract no. 07.0202/2016/740982/ETU/ENV.D2 and remain relevant:

IAS already dealt with elsewhere:

- The risk assessment for *C. finlaysonii* developed by the Bern Convention secretariat will be reviewed and updated under this contract.

- Ten of the terrestrial invertebrate species on the horizon scanning list are plant pests, such as *Agrilus planipennis*, and were therefore excluded because they are already in Annex I or II of the EU Directive regarding plant health (2000/29/EC).

- We also excluded species currently being assessed within the LIFE project Mitigating the threat of invasive alien plants in the EU through pest risk analysis to support the EU Regulation 1143/2014 (http://www/iap-risk.eu/) which has prioritised 16 plant species for risk assessment in 2016/17.

Congeneric and closely-related species:

During the first round of assessments in 2017 considerable discussion focussed on whether or not it would be possible (and permissible) to develop a risk assessment for more than one species (or even a whole genus) in cases where pathways, impacts etc. are similar or identical. The discussion focussed mainly around three ant species in the genus *Solenopsis* and the lion fish *Pterois miles* and *P. volitans*. In 2017 *Solenopsis invicta* was selected as one of the ten species for risk assessment given its well-documented invasive potential and impact, and considered together with two other species in the same genus (*S. geminata* and *S. richteri*). However, during assessment task three it became apparent that a single assessment was not appropriate, because of subtle but sufficient differences between the species. *Solenopsis geminata* and *S. richteri* are thus re-entered into the process for single species assessments. Indeed all the assessments suggested here will consider only single species.

Feasibility of management:

We took the view that the selection of species for risk assessment also needs to take into consideration the feasibility of cost-effective management. This was not a consideration in our original horizon scanning exercise, but becomes highly relevant when selecting species for risk assessment, particularly in relation to Article 4(e) of Regulation 1143/2104, according to which "it is likely that the inclusion on the Union list will effectively prevent, minimise or mitigate their adverse impact." In the case of many species, even those with high individual and combined scores for arrival, establishment, spread and impact, realistic management is unlikely for various possible reasons, including: population densities are too low/dispersed to allow effective control; the impacts of control measures on non-target species in the ecosystem are too severe; control measures are unlikely to be acceptable to the public. Many invertebrates were deemed to be near-impossible to eradicate once established and were not prioritised for this reason. However the *Solenopsis* species were selected because they are not yet present in EU and it would make sense to try to prevent their entry into EU.

It must be noted that the term ‘management’ beyond the definition under Regulation 1143/2014 covers multiple measures including early warning systems, awareness raising, legal enforcement of contained holding conditions and prevention of certain activities such as transport of soil from infested areas. Thus, some experts found it difficult to answer the question about whether management was feasible in a binary 'yes/no' manner without a clearly defined management objective.

Beyond these considerations about feasibility of management, the prioritisation ultimately is based on the potential impact of the species on biodiversity. Therefore, also species with already a wider distribution within the EU (and therefore potentially more difficult management) have been selected. A complete risk assessment and a dedicated effort to collect concrete information on the possible measures to deal with them was deemed important to inform policy decisions. In particular, it was the European Commission that requested the inclusion of *Cydalima perspectalis* in the final selection as this is a recurrent issue in the last years.

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), Dan Chapman (CEH), James Bullock (CEH), Beth Purse (CEH)

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 (HCMR), Jack Sewell (MBA), Marc Kenis (CABI), Piero Genovesi (IUCN), Niall Moore (NNSS), Olaf Booy (NNSS) plus other relevant experts.

Table 1. 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
Polygonum polystachyum (Himalayan Knotweed)	Rob Tanner Etienne Branquart	Oliver Pescott Johan van Valkenburg	Rob Tanner Peter Robertson
Solenopsis richteri (black Imported Fire Ant)	Olivier Blight	Wolfgang Rabitsch Jørgen Eilenberg Richard Shaw Marc Kenis	Olivier Blight Peter Robertson Richard Shaw

Solenopsis geminata (tropical fire ant)	Olivier Blight	Jørgen Eilenberg Richard Shaw Marc Kenis	Olivier Blight Richard Shaw Peter Robertson
Cydalima perspectalis (box tree moth)	Marc Kenis Wolfgang Rabitsch	Marianne Kettunen Gábor Vétek Archie Murchie	Marc Kenis Gábor Vétek Archie Murchie Jørgen Eilenberg Peter Robertson
Callosciurus finlaysonii (Finlayson's squirrel)	Sandro Bertolino Tim Adriaens Yasmine Verzelen Wolfgang Rabitsch Peter Robertson Marianne Kettunen Dan Chapman Riccardo Scalera	Craig Shuttleworth Sven Bacher Vinciane Schockert	Peter Robertson Tim Adriaens Sandro Bertolino Craig Shuttleworth
Xenopus laevis (African clawed frog)	Riccardo Scalera Wolfgang Rabitsch Piero Genovesi Tim Adriaens Yasmine Verzelen Peter Robertson Dan Chapman Marianne Kettunen	John Measey Sven Bacher	Riccardo Scalera Peter Robertson
Fundulus heteroclitus (mummichog)	Juan Diego Alcaraz-Hernández Emili García-Berthou	Wolfgang Rabitsch Quim Pou-Rovira Marianne Kettunen	Emili García-Berthou Juan Diego Alcaraz-Hernández Peter Robertson
Morone americana (white perch)	Luke Aislabie Hugo Verreycken Daniel Chapman Gordon Copp	Felipe Ribeiro Wolfgang Rabitsch	Hugo Verreycken Luke Aislabie Gordon Copp Peter Robertson
Perna viridis (Asian Green mussel)	Jack Sewell Paul Stebbing Phil Davison	Marika Galanidi Argyro Zenetos	Jack Sewell Paul Stebbing Phil Davison Peter Robertson
Lagocephalus sceleratus (silver-cheeked toadfish)	Marika Galanidi Argyro Zenetos Daniel Chapman	Jack Sewell Elena Tricarico Wolfgang Rabitsch	Marika Galanidi Argyro Zenetos Jack Sewell Elena Tricarico Peter Robertson

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

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.

Polygonum polystachyum = *Koenigia polystachya* (Himalayan knotweed)

The result of the risk assessment indicated that the species risk is moderate, with a medium level of confidence. In the risk assessment two introduction pathways have been identified (1) horticulture (escape from confinement), and (2) transport – contaminant (transport of habitat material (soil and vegetation). However, the former pathway is likely to be a historic pathway for entry into the RA area. To-date, the species has been recorded in the following biogeographical regions Atlantic, Alpine, Boreal and Continental, and, the species has the potential for further establishment in the aforementioned biogeographical regions and the Mediterranean region. In its introduced range, it has a moderate impact on biodiversity and related ecosystem services, including additional impacts on the economy and provides no benefits. The reflection of the medium confidence is mainly a result of the lack of scientific studies detailing the impact of the species in the introduced range. In fact, at this current time, there are no known scientific studies evaluating the impact of the species.

Summary of key points in response to the peer-review

1) Risk Assessment

One review highlighted that the risk assessment had detailed other invasive knotweed species that looked similar to *K. polystachya* but in their view none of the species detailed would be confused with *K. polystachya* by a competent field botanist. The text was amended to reflect this comment. Further suggestions were made on the structure and editorial changes which were incorporated into the final version.

A further review questioned the overall likely score for the pathway horticulture (escape from confinement), highlighting that this is more of a historic pathway and suggested the score should be reduced to moderately likely, this was incorporated into the final document. In addition, the overall score of rapidly for spread was queried and it was highlighted that spread is often slower and thus the overall score was reduced to moderately. In addition, due to the lack of scientific publications on the impact of the species it was suggested that the uncertainty for impacts should be changed from moderate to low and again this was included in the final document.

2) Template for Annex with evidence on measures

Following the reviewers comments a number of amendments were made:

- For consistency each method was detailed in a separate row with its own confidence assessment,
- Education and awareness raising was added to the section 'methods to achieve prevention'.

- Costs were added for management measures – these costs were taken from costs of controlling *Fallopia japonica* as there are no estimate of costs for controlling *K. polystachya*.

***Solenopsis richteri* (The Black Imported Fire Ant)**

1) Risk assessment

The species is probably one of the least successful invasive ants. Due to the limited distribution of *S. richteri* in the USA, there is much more information available on the biology and ecology of *S. invicta*. Several questions have been answered with information on this former species. This point was the subject of extensive expert discussion because it increased the uncertainty of the risk assessment so in some cases the level of confidence was reduced when specific data on *S. richteri* was lacking. The peer-reviewers also highlighted some inconsistencies in the scores when compared alongside the other ant assessed in this round *S. geminata*, leading to some adjustments.

There is moderate evidence that *S. richteri* is, and will be, able to enter and establish in the risk assessment area. There is also moderate evidence that the species will be able to spread across suitable habitats. However, a certain level of uncertainty in its predicted distribution under both current and future climatic conditions was noticed, as the assessment was based on one species-distribution model. It has been highlighted that any future application of additional models would improve the prediction and confidence level of the assessment.

The two points above were the main issues raised by the peer-reviewers. The outcomes of the discussions were included within the revised text during the workshop held in Brussels on October 11-12 2018.

2) Template for Management Annexe with evidence on measures

The Management Annex was also subjected to improvements, particularly by adding clarification to the discussed measures of control (e.g. data on biological control using parasitic flies and entomopathogenic agents). In most cases all inputs were incorporated in the text, and were validated during the workshop held in Brussels on October 11-12 2018.

***Solenopsis geminata* (tropical fire ant)**

1) Risk assessment

Despite that the species is probably the most widely distributed invasive ant, its impacts are considered moderate compared to other ant species. There is evidence that *S. geminata* is, and will be, able to enter and establish in the risk assessment area. There is also evidence that the species will be able to spread across suitable habitats. However, a certain level of uncertainty in its predicted distribution under both current and future climatic conditions was noticed, as the assessment was based on one species-distribution model. It has been highlighted that any future use of additional models would improve the prediction and confidence level of the assessment. This was the main issue raised by the peer-reviewers.

In a few questions the scores and their level of confidence were also subject to expert discussion. The peer-reviewers highlighted some inconsistencies in the scores and associated levels of confidence, leading to some adjustments. The outcomes of the discussions on the points above were included within the revised text during the workshop held in Brussels on October 11-12 2018.

2) Template for Annex with evidence on measures

The management annex was also subjected to improvements, particularly in adding clarification to the actual feasibility and cost of the discussed measures (e.g. data on biological control using parasitic flies). In most cases all inputs had been incorporated in the text, and were validated during the workshop held in Brussels on October 11-12 2018.

Cydalima perspectalis **(box tree moth)**

The result of the risk assessment indicated that the species risk is high, with a high level of confidence. This is because it is already present in most of its potential range, which covers most natural stands of native *Buxus* spp. in Europe. If no area-wide management method is implemented to lower populations in natural stands, e.g. through the introduction of a specific natural enemy from Asia, or if no resilience of *Buxus* spp. stands are observed in the next few years, the risk is high that whole ecosystems will disappear, including many species that live exclusively in these ecosystems.

Summary of key points in response to the peer-review

1) Risk Assessment

In general, comments from the two reviewers were rather minor and consisted mainly in corrections of typing errors and changes in the vocabulary, as well as suggestions for references and, more importantly, suggestions for changes in a few scores and confidence levels. These were discussed within the expert group and some scores were modified. An important issue raised by one reviewer was discussed at the workshop. It was suggested to provide details on the presence or absence of *C. perspectalis* in the Balearic Island and Sardinia, where a few stands of the rare *Buxus balearica* occur. Data were provided in this matter, as well as consequences for management in Annex 4.

2) Template for Annex with evidence on measures

Following the comments mentioned above on Mallorca (where the moth is absent) and Sardinia (where it is present but where it is not known if it has already reached the rare *B. balearica* stands), suggestions for measures to protect *B. balearica* on these two islands were added. One reviewer suggested adding light trap for monitoring. Another review suggested removing the word "biopesticide" and considering all methods involving the use of products based on insect pathogens as "biological control". Finally, another reviewer requested more quantitative data on the costs of management methods. These were provided where possible.

Callosciurus finlaysonii **(Finlayson's squirrel)**

The species is already established in the risk assessment area following deliberate releases and escapes. As the zoo and pet pathways are still active and the current populations represent a potential source of entry/translocation/natural dispersal to other parts of the RA area, it is likely to enter other parts of the RA area. After extensive discussion on the potential for squirrels to escape, reference was made to (1) red squirrel escapes (totally unintentional) from woodland enclosures in captive Zoological collections (2) similar escapes of other *Callosciurus* spp. in the RA area (3) evidence that only a limited number of founders is required for successful establishment of *Callosciurus* spp. as an indication of the potential for entry through the pathways considered. Nevertheless, confidence was set at medium as information on the number of squirrels sold, kept as pets or kept in zoos is scarce. The level of uncertainty on the SDM was high due to a small number of distribution records available, possibly not capturing the full range of conditions in which the species can establish. However, the model did suggest suitable areas for establishment in the RA area and this is corroborated by successful establishment of closely related species in those regions. Also, it was acknowledged squirrels can escape limiting climatic conditions through behavioural adaptations with reference to supplemental feeding in urban areas which facilitated establishment of the tolerant and adaptable *C. finlaysonii* in Italy. Hence, despite uncertainties in the SDM, establishment outside the already occupied area was scored very likely with high confidence. There was some discussion around the potential for unaided spread, for which quantitative data are available from range expansion in Italy, and amplification of spread by human intervention. It was concluded that, regardless the initial lag time the species clearly has dispersal capacity, but this depends on the landscape configuration hence spread potential was considered moderate. Discussion was held around potential ecological impact, as currently quantitative data on this impact category are largely lacking and inferred from studies on other species. It was noted the species is now occurring sympatric with native red squirrel, and there is certainly potential for impact on Calabrian black squirrel *Sciurus meridionalis*, an endemic species of conservation concern with a very restricted range. This was emphasized more in the risk assessment following discussions at the workshop.

Summary of key points in response to the peer-review

1) Risk assessment

During the workshop, risk assessment scores and confidence levels were revised by the group, thereby focusing on the risk assessment modules where confidence was deemed low, more specifically the entry, spread an impact modules. The group also identified some missing scores (1.9c, 1.10c). Published evidence (e.g. on trade, data on squirrels kept in zoos, potential for escape of other squirrel species) was added to support statements on entry potential through the pathways escape and release (e.g. 1.4c, 1.10b) and the text on prices in sale was adapted with regards to new information on squirrels in trade. Information was added on potential ecological impact in relation to native species presence, as well as evidence on health risks of Bornovirus. It was stressed that in this case, emphasis is on scoring the pathways of entry rather than the species. Following exchanges with assessors of other taxa, revision of spread and establishment modules were carried out stressing establishment in the wild and the probability of the species to establish and spread outside already invaded areas in the RA area (with spread between member states being mentioned more explicitly) and this was reported back to the entire group as a potentially important point for template clarification. Similarly, spread should not include anthropogenic repeated releases. Further discussions around the RA template included the use of the term moderate, the definition of the scoring range for impact between moderate and major with regards to extinction and reversibility, the coverage of spread in the scoring categories (rapidly-slowly),

In conclusion, the peer-review process highlighted a few inconsistencies in the scores and associated confidence, leading to some adjustments. Extra references were added to support the statements made. Furthermore, some smaller textual changes were made such as correct reference to legislation and better question formulation in the spread module, improving the general quality, consistency and readability of the text. The outcomes of the discussions on the points above were included within the revised text following extensive discussion during the workshop held in Brussels on 11-12 October 2018.

2) Template for Annex with evidence on measures

The management annex was discussed and revised, but overall, with good information available on established methods for squirrel control, the quality of the information provided was considered good by the reviewers and the group. Information was added on the potential of surgical sterilization, already practiced in the risk assessment area, as a management method as an alternative to lethal control of small populations in urban areas or small areas where sensitivity to animal welfare is high. Also, disposal of the animals was more explicitly mentioned as this incurs costs. These changes have been embodied in the text following discussions held during the workshop in Brussels on 11-12 October 2018.

***Xenopus laevis* (African clawed frog)**

The species is known to have a clear impact in the risk assessment area, and there is evidence that further releases (or spread) may occur in areas not yet colonised (mostly as a consequence of the species being kept in research labs and as a pet). A certain level of uncertainty in the SDMs was noticed, as it was made on the basis of datasets from previous works, using different approach (it was clarified that the ensemble model may be considered the most reliable). There was extensive discussion on the level of impact of the species, which is mostly due to the predation on macro-invertebrates communities and possibly on some vertebrates (including species protected by the Habitats Directive). The impact as potential vector of the chytrid fungus, often considered as the main threat related to the species, was probably overestimated and in any case is not yet documented, hence was considered minor.

Summary of key points in response to the peer-review

1) Risk assessment

The peer-review focussed on three key aspects: the current knowledge of the species distribution and taxonomy, the impact of the species (particularly in relation to spread of the chytrid fungus) and the actual feasibility of some management options. The categorization of scores and level of confidence was also a central element of discussion. Overall the peer-review was valuable in clearly formulating many aspects of the discussion from an earlier version of the risk assessment, particular thanks to the specific expertise of a peer reviewer, who is also one of the leading authors of many well-cited papers on the target species. One example of considerable discussion through the peer-review was in relation to concerns about the much discussed impacts of the species as a potential vector of the chytrid fungus (never demonstrated in practice, despite ambiguities in the literature) and other pathogens and diseases. The peer-review also contributed to clarification of some important aspects of the species taxonomy and distribution, in addition to its ecology (for example, in relation to movements and spread). Finally, the peer-review process highlighted some inconsistencies in the scores and relevant level of confidence, leading to some adjustments. The outcomes of the discussions on the points above were included within the revised text following extensive discussion during the workshop held in Brussels on 11-12 October 2018.

2) Template for Annex with evidence on measures

The management annex was also subjected to several improvements, particularly in relation to the actual feasibility and effectiveness of the discussed measures, which in some cases were not very clear in the literature. In most cases all inputs have been embodied in the text, and were further discussed during the workshop held in Brussels on 11-12 October 2018.

***Fundulus heteroclitus* (mummichog)**

The mummichog is a cyprinodontiform fish native to eastern coast of North America, where it is very abundant. It is used in the aquarium hobby and for research and could entry through these and other pathways. It is a very hardy species that tolerates a range of temperatures and salinities, has established in two separate areas of the Iberian Peninsula and it is very likely to establish in most coastal areas of the European Union, if introduced. It is rather a sedentary species that has been shown to spread in the Iberian Peninsula although infrequently and slowly. It seems to already impact endemic, endangered Iberian cyprinodontiforms, with less impacts in ecosystem services and reduced economic costs. If introduced to other Mediterranean areas, it is likely to impact other endemic fauna. Therefore, the overall result of the risk assessment indicated that the species risk is high, with a medium level of confidence.

Summary of key points in response to the peer-review

1) Risk Assessment

The three peer-reviews focused on general edits, clarifications on some points, questions about some pathways and ecosystem services. Specifically, one reviewer disagreed with the general sentence "introduction pathways are unclear" explaining that at least for the Ebro Delta the pathway is most likely to relate to introduction from a research institute. Therefore, the text was revised but noting that most of the published references (e.g. Gisbert & López, 2007, Morim et al. 2018) are open about the possible pathways and do not discount several possibilities. There was an additional query on the origin of this species within the Ebro Delta from South Spain; a recent paper (Morim et al. 2018) was included that shows that there is virtually no genetic diversity and distance among Iberian populations. The reviewer also outlined a personal observation that the irregular use as a bait fish species, and its implication for its dissemination to other regions of EU noting that this was practise amongst anglers fishing on amphidromous or marine species (ie *Dicentarchus labrax*) in the Ebro Delta. Additionally it was noted that at least two other species with first citations in Spain in the Ebro Delta, *Misgurnus anguillicaudatus* and *Pseudorsabora parva*, are now quickly expanding in Catalonia and other parts of Spain mainly due to its irregular use as bait fish.

2) Template for Annex with evidence on measures

The focus of the review was on reducing the use of highly technical terms and so increasing overall clarity. Additionally, to separate possible measures and provide separate confidence assessments for each. Furthermore, the reviewer requested clarification on the units and scale of economic costs and to add further information on potential methods to achieve management. All comments were addressed as fully as possible.

***Morone americana* (white perch)**

The risk assessment indicated that white perch has an overall moderate risk, with a medium level of confidence. This species is not yet in the RA area and it is considered unlikely to enter the EU (medium confidence) but establishment is likely in most parts of the RA area when introduced (medium confidence). Spread and impact are considered to be moderate with medium to low confidence. Main focus must be on preventing the white perch entering the EU.

Summary of key points in response to the peer-review

1) Risk Assessment

The first referee commented on the inclusion of *Morone* hybrids in the risk assessment. The authors wanted to draw the attention on these hybrids as one of these hybrids (*M. chrysops* · *M. saxatilis*) is already in the RA area. This was clarified in the text. Textual suggestions were generally followed and CBD terminology for pathways was used as suggested. A few scores and their level of confidence were adjusted to the referee's suggestion.

The second referee added an important reference (book chapter) on the distribution and the ecology of white perch, the referee's additions hereon were all incorporated in the risk assessment. Also most of the textual additions were taken in. A few scores and their level of confidence were adjusted to the referee's suggestions.

2) Template for Annex with evidence on measures

The reviewer added parts of text and suggested some rewording and deleting of other parts of the text; all these comments were accepted. The use of eDNA was added as an early detection method. As suggested, the levels of confidence were all checked to be sure they reflect the confidence in the content of the assessment and not the confidence in the effectiveness of the methods. Where necessary, levels were adjusted. It was clarified in the annex at what scale the costs were estimated and it was stressed in the text that the estimates are very rough estimates with low or no support from published cases. The 'Methods to achieve management' section was rewritten to better clarify the difference with the methods used for eradication.

***Perna viridis* (Asian Green mussel)**

The results of the risk assessment indicate that the risk posed by this species to the EU is high, with a medium level of confidence. *Perna viridis* exhibits a number of traits, which have led to successful introduction, establishment and spread globally. Although not yet recorded with EU waters, these traits, combined with the presence of potential pathways of entry, make introduction likely throughout the Mediterranean and on the Atlantic coast of Spain and Portugal. Once established, environmental, economic and social impacts are likely to be high, with particular impacts on native sessile organisms and a range of habitats.

Summary of key points in response to the peer-review

1) Risk Assessment

All minor changes and amendments made by the reviewers were accepted.

There was some discussion with reviewers as to whether an annex containing the extensive information on physiological requirements should be created; it was decided that this information would be included in the main body of text as required.

The pathway under question 1.2 has been changed to release in nature: fishery in the wild, following CBD classifications. A reviewer suggested the use of CBD classifications of 'unaided' for natural dispersal, but as different subcategories were discussed in the risk assessment this suggestion was not adopted to ensure clarification of the pathway being discussed.

A reviewer suggested the inclusion of separate risk scores for different regions and subregions of the risk assessment area. While we agree with the idea, the current framework does not facilitate this approach and therefore it has not been taken up. The Member States in the English Channel and the Celtic Sea into which the species could establish under future climatic conditions have been clarified.

The reproductive strategy of the species and the differences that may occurring across the risk assessment area has been clarified throughout the risk assessment.

A reviewer asked if there was additional information available on the species interfering with recreational activities or infrastructure. The authors were unable to find any such additional information.

Additional climatic maps have been added using the Copernicus platform.

2) Template for Annex with evidence on measures

A number of minor comments were addressed in the final version.

***Lagocephalus sceleratus* (silver-cheeked toadfish)**

Lagocephalus sceleratus is a lessepsian fish species that has become notorious in the eastern Mediterranean as a fisheries pest. It is a fast-spreading species that has reached the south-western Mediterranean within approximately 10 years; further spread in areas of the Mediterranean not colonised so far is predicted by the SDM but spread to the Atlantic and the Black Sea is expected to be limited. The socio-economic impacts are severe and well documented; that is not the case however for the environmental impacts, which are suspected to be high but the evidence is scant. This was the main point of discussion during the reviewing process.

Summary of key points in response to the peer-review

1) Risk Assessment

The reviewers' comments were constructive, helpful and in good spirit and helped us improve the assessment and provide more comprehensive content where needed.
At the early stages of the reviewing process we were asked by the reviewers and the European Commission to add the CORRIDOR pathway (Suez Canal) in the pathways of introduction and also assess it in the Management Annex, which we promptly did. Even though the species will not enter the risk assessment area via this pathway, the pathway is still active and will very likely result in repeated introductions which may increase the genetic diversity of the invasive populations with implications for further adaptability, spread and population control efforts. We were also asked to add a comment on the reasons why we did not include ballast water transport as a pathway of introduction; this is included in Q1.1. Information on the potential use of the species in the ornamental aquarium trade was requested from the Ornamental Aquatic Trade Association (OATA), whereby we were informed that *L. sceleratus* is not in the aquarium trade (Tracey King, pers. comm.). Additionally, the European Union Aquarium Curators (EUAC) provided

information on the presence of the species in public aquaria; accordingly, the pathway ESCAPE (from aquaria) received a low score as a potential pathway of introduction.

Additional information was requested (and provided) on the potential for predation control of *L. sceleratus* and some questions were raised with respect to the adaptability of the species to cooler temperatures than those experienced in the native range. It appears that isolated records of the species exist in waters with rather cold temperatures (i.e. records below the 16°C threshold used in the model as a limit for establishment); thus, some populations may already be tolerant to lower temperatures than this threshold. Further adaptability cannot be discarded and is actually considered quite likely.

The biggest challenge in the *L. sceleratus* risk assessment was the quantification of environmental impacts with appropriate data. This was pointed out by all the reviewers and we made every effort to provide as much information as possible to clarify our conclusions and support our assessment. Indeed, there is a lack of quantitative data that unequivocally demonstrate a cause and effect relationship between increasing populations of *L. sceleratus* and the decline in the populations of preferred prey species. It is repeatedly acknowledged in the relevant sections that the assessment of impact is based on correlative data with fisheries landings in combination with prey preference information and anecdotal reports from fishermen, and this increases the uncertainty for the given scores; this is also reflected in the low confidence levels for the majority of the environmental impact scores. We also sourced and present standardised data (Catch per Unit Effort – N. Michailidis, DFMR, Cyprus - and Catch per Unit Area data from the literature) in response to reviewers' comments that landings data may not be appropriate as a measurement unit for population fluctuations (either of *L. sceleratus* or of potential prey species). With respect to the potential impact on the conservation value of *Posidonia oceanica* meadows, a clarification was added that conservation value will be threatened primarily through predation and this will be more severe in habitats that are used as nursery grounds for fish and invertebrates, such as seagrass beds. Structural impacts are not anticipated.

2) Template for Annex with evidence on measures

A comment was made regarding commercialization of the species as a means of population control, that any such measure should be carefully implemented as it may introduce conflicting management objectives for a sustainable fishery and "institutionalize" the invasive species.

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:

Adriaens, Tim	Kenis, Marc	Shuttleworth, Craig
Zenetos, Argyro	Measey, John	Stewart, Alan
Bacher, Sven	Murchie, Archie	Tanner, Robert

Bertolino, Sandro
Blight, Oliver
Copp, Gordon
Eilenberg, Jorgen
Galanidi, Marika
García-Berthou, Emili
Peyton, Jodey
Roy, Helen
Scalera, Riccardo
Sewell, Jack
Shaw, Richard
Tricarico, Elena
van Valkenburg, Johannes
Verreycken, Hugo
Verzelen, Yasmine
Vetek, Gabor

Apologies:
Kettunen, Marianne
Robertson, Peter
Schonrogge, Karsten
Pou, Quim

Programme

Day 1 (11th October 2018)

Time	Agenda Item	Lead	Paper
930	Arrival – coffee in canteen		
945	Welcome and introductions	EC	Oral
1000	Overview of the project	Helen Roy	Oral
1015	Overview of the risk assessments (10 minutes talk + 10 minutes discussion per assessment)	RA lead authors and peer reviewers	Oral
1330	Wrap up of morning session	Helen Roy	Oral
1335	Lunch		
1415	Breakout groups to finalise risk assessments	Groups	
1730	Close for the day		

Day 2 (12th October 2018)

Time	Agenda Item	Lead	Paper
930	Arrival		
930	Feedback on breakout groups to finalise risk assessments	Helen Roy	Oral
1130	General discussion: risk assessment template	Helen Roy	Oral
1245	Lunch		
1345	Management annex	Pete Robertson	Oral
1400	Groups finalise management annex	Groups	

1545	Summary and next steps	Helen Roy	Oral
1600	**Close and depart**		

The agenda was designed to be flexible but began with a short introduction from the European Commission followed by an 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.

Following the workshop discussion, experts were asked to complete all risk assessments noting the confirmation of the text for question 2.9, 2.11, and 2.12, as follows:

- 2.9 a,b,c etc Estimate the potential rate of spread within the Union based on this pathway (please provide quantitative data where possible)
- 2.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues and please provide quantitative data where possible).
- 2.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (please provide quantitative data where possible)

Note Q2.1. also covers this area but is not pathway specific

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

The experts were also informed that:

- all photos and figures within the risk assessment must be free from copyright restrictions.
- any further improvements to the template noted during the workshop will be discussed at the kick off meeting on 12 December – including improved guidance on i. pathways in relation to introduction and spread questions; ii. assigning confidence

Workshop discussions on the risk assessments

During the workshop held in Brussels on 11-12 October 2018, the issues raised through the peer-review process were discussed in detail, and contents of key references were assessed, particularly in relation to the species impact. Following agreement between all participating experts on the actual impact of the species, there was additional discussion on the scores and level of confidence to be attributed. In particular, it was noticed that as there is no evidence of the impact leading to any irreversible change, then it is to be considered "moderate". The participants however agreed that the term "moderate" did not seem the most appropriate at conveying the correct message.

As a key remark, to be considered also for further improvement in the near future, is the difference pointed out between the categories used in the risk assessment (as defined in Annex II) and those adopted by EICAT and other works quoted in the risk assessment itself. This is due to the fact that according to EICAT a major impact is still reversible, while in Annex II a major impact is considered irreversible. Hence whatever is considered as a "major" impact in EICAT should be considered as "moderate" in the risk assessment. This apparent inconsistency was discussed clearly in the risk assessment to help the reader understand how scores are applied and prevent possible confusion on the issue.

Clarifications were provided by the European Commission in relation to the pathways categorisation system. Attention should be paid to the fact that Delegated Regulation (EU) 2018/968[3] requires to use as basis the CBD classification[4]. Furthermore, it requires to address pathways of "introduction" and "spread", including "the ability of transfer from those pathways to a suitable habitat or host". The risk assessment template used differentiates between "introduction" and "entry" with the latter corresponding to "ability of transfer from those pathways to a suitable habitat or host".

The CBD category (1) release in nature is to be read as intentional and the rest (2)-(6) as unintentional, e.g. 1. introduction in the risk assessment area, is to be considered intentional in the case of the animals being imported for the pet trade and use in scientific research; 2. Subsequent entry into the environment, can be either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes. This led to the need to cover a number of points in the risk assessment which were initially disregarded as per instruction because they were not deemed relevant to intentional introductions. This is an element to clarify and improve in the future version of the template.

Another issue discussed during the workshop, and which should be clarified within the risk assessment template for the next phase of the contract, is the use of the level of confidence. This usually refers to the quality of information source rather than on the quality of information itself (e.g. experts may not always agree with what's stated in scientific papers), which of course may lead to some confusion. Instructions should be adjusted taking into account that Delegated Regulation (EU) 2018/968 requires that the level of confidence attached to the answers in risk assessments shall reflect the possibility that information needed for the answer is not available or is insufficient or the fact that the available evidence is conflicting.

[3] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2018:174:FULL

[4] UNEP/CBD/SBSTTA/18/9/Add.1

Workshop discussion on the management annex

Assessment and discussion of the management annexes with the European Commission at the workshop included the following point within the context of three different objectives:

1. To describe the available methods for prevention, eradication and on-going management.

The current approach is considered to effectively broadly identify appropriate methods.

In many cases methods for prevention are generic (treat ballast water, enforce existing legislation etc) rather than species-specific.

It may be better to produce a limited number of texts on these generic prevention methods rather than repeatedly produce short accounts for individual species.

In most cases, the assessments recommend a combination of available methods rather than the use of any one in isolation.

2. To consider the feasibility and effectiveness of methods.

The effectiveness, feasibility and costs of management are all known to be strongly scale dependent, but the available literature rarely considers the effects of scale on these measures.

Some information on the feasibility of eradication at different scales is available from the literature.

For long-term management, any assessment of the feasibility and effectiveness of different methods is complicated by the multiple objectives of management (to reduce spread, damage and/or abundance) which are often not clearly described in the literature, as well as the effects of scale.

Other methods, such as the use of expert elicitation, may provide a route to supplement the available evidence (for eradication) or to better assess feasibility and effectiveness in relation to specific objectives (for long-term management) but this is beyond the scope of this study.

3. Assess the costs and/or effort required by the methods

Information on management costs and effort from the literature is often very limited.

Management effort is more widely reported in the literature than cost.

Costs and effort are presented in a wide variety of formats in the literature, but need to be per unit area and per unit time to be comparable. They are rarely presented in this way.

Costs and effort are more widely reported for prevention and eradication, where the objectives are straightforward, than for long-term management where the objectives are often opaque.

Other methods, such as the use of expert elicitation of the costs and effort required to achieve different objectives at different scales using methods in isolation or in combination, may be more useful than relying on the limited published literature on management costs.

There was some confusion in relation to the level of confidence, as it may seem to refer to the effectiveness/feasibility of the method, rather than on the underlying information sources. Streamlining between the 10 annexes was done in the final versions and related instructions will be strengthened over the next year.

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. Following discussion of last year's project, a new summary section was added the Annex, asking the authors to summarise the main findings and the most appropriate methods to consider at different stages.

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 each method it was recommended that the annexes include an assessment of the likely cost and effectiveness. Where information was available, the following range of questions were suggested for consideration, accepting that not all questions would 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 was provided. When describing case studies, information on both total cost and the area over which control was undertaken was extracted so that a cost per unit area might be derived. Where such quantitative information was not available, then any qualitative information from the literature was acceptable to help guide decision making. It was accepted that in the majority of cases the information required to assess the potential total cost of management at a Member State level was unlikely to be available. This would normally require information on the extent and abundance of the species which was beyond the scope of this assessment. Assessors were not expected to extrapolate the potential total costs of management at a Member State level, but only to report on the information provided within the literature.

The measures documented in the annexes were categorised in terms of:

- prevention: methods that might be applied by Member States to support prevention: i.e. preventing a species entering by blocking its pathways. This assumed that the Member State was free of this species.
- eradication: 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.
- population control: methods that might be applied by Member States to support population control, for example 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.

Issues and key recommendations

Most of the issues and discussion points arose with reference to the implementation of the risk assessment template (Task 3). Here we provide a summary of these issues and key recommendations.

Level of confidence: This usually refers to the quality of information source rather than on the quality of information itself (e.g. experts may not always agree with what's stated in scientific papers), which of course may lead to some confusion. For example, it could be clarified that the level of confidence should relate to the quality of information on the assessment (hence encompassing literature sources and expert opinion, although this may lead to the risk of the confidence level being overly subjective).

Instructions for the level of confidence in the risk assessment template regarding confidence should be further clarified, taking into account the Delegated Regulation (EU) 2018/968. This requires that "Each answer provided in the risk assessment shall include an assessment of the level of uncertainty or 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".

Also, in the management annex confusion may also exist in relation to the level of confidence whereby it might be used to refer to the effectiveness/feasibility of the method, rather than on the underlying information sources. For each method described in the risk management annexes, an overall assessment of the confidence that could be applied to the information was given. Following feedback on last year's project, the wording of the advice in this section will be strengthened to emphasise that this confidence relates to the quality of the available information and not 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.

Where there were further factors beyond these that determined the chosen level of confidence, a brief written description to support the choice of the level of confidence was provided.

Categories of impact: a difference was pointed out between the categories used in the risk assessment (as defined in Annex II) and those adopted by EICAT and other works quoted in the risk assessment itself. This is due to the fact that according to EICAT a major impact is still reversible, while in Annex II a major impact is considered irreversible. Hence whatever is considered as a "major" impact in EICAT should be considered as "moderate" in the risk assessment. Authors using the template should be made aware of these differences and follow the definitions as in Annex II.

Pathways categorisation: compliance with the recently approved delegated act, which requires pathways to be discussed in terms of: I. introduction in the risk assessment area, which is to be considered intentional in the case of the animals being imported for the pet trade and use in scientific research; II. entry into the environment, which can be either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes. This led to the need to cover a number of points in the risk assessment which were initially disregarded as per instruction because not relevant to intentional introductions.

There was discussion about pathways related to human mediated releases in the "probability of Spread" section, although they were explicitly excluded (likely to avoid repetitions and redundancies with the section "probability of introduction and entry"). This aspect needs to be clarified with the EC for the third year of the contract.

Template structure: the use of tables was not considered practical by all authors, as it seems to take a lot of space, and does not allow an easy management of the track changes of several authors across different versions. A plain text, with a heading for each question, followed by a subheading representing the "instruction", with a line in bold to indicate the score and level confidence, would represent an alternative option to consider for the third year of the contract.

Species Distribution Models (SDMs): this section requires a lot of work and sometime interpretation of results may be challenging in relation to quality of underlying dataset, which is not always easy to judge given the many constraints and knowledge gaps about, for example, taxonomy and distribution. However, such a modelling approach was seen as useful by many authors for guiding responses.

Management annex: No changes were suggested, except for clarification on the level of confidence. Inclusion of comments should be considered regarding an additional horizontal component of the control/eradication measure for example additional information on the use or disposal of animals and plants removed from the environment.

In the majority of cases, the recommended management approach involves the use of multiple methods in combination. These combined-method approaches are not explicitly considered by the current individual method-based accounts. This aspect should probably been given more visibility in the annex.

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, five were deemed to constitute a "high" risk (Table 3). For four of the five species deemed "high" risk the confidence was "medium" and for one species (*Cydalima perspectalis*) the confidence was given as "high". For four of the five species deemed "moderate" risk the confidence was "medium" and for one species (*Solenopsis richteri*) the confidence was given as "low". 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 3. Compilation of the responses (High, Moderate, Low) and confidence (High, Medium, Low) assigned within the conclusion of the risk assessments

Species	*Response*	*Confidence*
Polygonum polystachyum (Himalayan Knotweed)	Moderate	Medium
Solenopsis richteri (black Imported Fire Ant)	Moderate	Low
Solenopsis geminata (tropical fire ant)	Moderate	Medium
Cydalima perspectalis (box tree moth)	High	High
Callosciurus finlaysonii (Finlayson's squirrel)	High	Medium
Xenopus laevis (African clawed frog)	Moderate	Medium
Fundulus heteroclitus (mummichog)	High	Medium
Morone americana (white perch)	Moderate	Medium
Perna viridis (Asian Green mussel)	High	Medium
Lagocephalus sceleratus (silver-cheeked toadfish)	High	Medium

References

Bacher, S. et al. (2018). Socio-economic impact classification of alien taxa (SEICAT). *Methods in Ecology and Evolution*, 9, 59-168

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2[5]
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 This risk assessment has been peer-reviewed by two independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study. **Date of completion:**

General instructions:

- Completing risk assessments can be time consuming. Risk assessors are guided to 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

[5] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

notifications and information from databases, including information collected through citizen science. All sources shall be acknowledged and referenced.

- 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.
- 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.
- Authors should not consider questions without specific instructions or explanatory comments less important as these are sufficiently self-explanatory. In case of doubt or uncertainty, authors may contact wolfgang.rabitsch@umweltbundesamt.at for clarification.
- 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	
Organism Information	**RESPONSE**
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.
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 wild, 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
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment 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
A5. What is the global non-native distribution of the organism outside the risk assessment area?	

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: Recorded: List regions [delete as appropriate] 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. Established: List regions [delete as appropriate] 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. Use the comments section to list 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).
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: Current climate: List regions 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.
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: Recorded: List member states [delete as appropriate] 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 Established: List member states

	[delete as appropriate] 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.
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: Current climate: List member states 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.
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?	
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	[delete as appropriate] 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 Indicate the area endangered by the organism as detailed as possible.
A12. In which EU member states has the species shown signs of invasiveness?	[delete as appropriate] 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 Indicate the area endangered by the organism as detailed as possible.
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.

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."
- The classification of pathways developed by the Convention of Biological Diversity shall 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].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	none very few few moderate number many very many	low medium high	Comment if entry pathways (i.e. pathways responsible for the occurrence in the wild) are different and list them.
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins	[insert text]		In this context a pathway is the route or mechanism of introduction of the species.

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

and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.			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).
Pathway name:	[insert pathway name here]		
1.3. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional unintentional	low medium high	
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely unlikely moderately likely likely very likely	low medium high	including the following elements: • 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 and entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction and entry whereas for others high propagule pressure (many thousands of individuals) may not.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely very likely	low medium high	including the following elements: • likelihood of survival, or reproduction, or increase during transport and storage;
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely likely	low medium high	

	very likely		
1.7. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely likely very likely	low medium high	• discuss the ability and likelihood of transfer from the pathway to a suitable habitat or host
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely moderately likely likely very likely	low medium high	
End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	very unlikely unlikely moderately likely likely very likely	low medium high	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.
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	very unlikely unlikely moderately likely likely very likely	low medium high	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 entry (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.

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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	very isolated isolated moderately widespread widespread ubiquitous	low medium high	
1.16. 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 ?	NA very unlikely unlikely moderately likely likely very likely	low medium high	
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very unlikely unlikely moderately likely	low medium high	

	likely very likely		
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	very unlikely unlikely moderately likely likely very likely	low medium high	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.
1.23. How likely is the adaptability of the organism to facilitate its establishment?	very unlikely unlikely moderately likely likely very likely	low medium high	

1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very unlikely unlikely moderately likely likely very likely	low medium high	
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	very unlikely unlikely moderately likely likely very likely	low medium high	
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	very unlikely unlikely moderately likely likely very likely	low medium high	
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	very unlikely unlikely moderately likely likely very likely	low medium high	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.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	very unlikely unlikely moderately likely likely very likely	low medium high	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.

PROBABILITY OF SPREAD

Important notes:
- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	minimal minor moderate major massive	low medium high	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
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	minimal minor moderate major massive	low medium high	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.
2.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways.	[insert text]		including the following elements:

For each pathway answer questions 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.			• 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:	[insert pathway name here]		
2.3. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional unintentional	low medium high	
2.4. 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?	very unlikely unlikely moderately likely likely very likely	low medium high	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).
2.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)?	very unlikely unlikely moderately likely likely very likely	low medium high	including the following elements: • likelihood of survival, or reproduction, or increase during transport and storage;

Subnote: In your comment consider whether the organism could multiply along the pathway.			
2.6. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely likely very likely	low medium high	
2.7. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely moderately likely likely very likely	low medium high	
2.8. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely very likely	low medium high	• the ability and likelihood of transfer from those pathways to a suitable habitat or host), including, where possible, details about the specific origins and end points of the pathways;
2.9. Estimate the overall potential for spread within the Union based on this pathway?	very slowly slowly moderately rapidly very rapidly	low medium high	
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	very easy easy with some difficulty difficult very difficult	low medium high	
2.11. Estimate the overall potential for spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	very slowly slowly moderately rapidly very rapidly	low medium high	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.
2.12. Estimate the overall potential for spread in relevant biogeographical regions in foreseeable climate change conditions	very slowly slowly	low medium	Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change

	moderately rapidly very rapidly	high	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 spread (e.g. increase in average temperature) 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 spread within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided.

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is 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?	minimal minor moderate major massive	low medium high	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
2.14. 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)?	minimal minor moderate major massive	low medium high	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.
2.15. 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?	minimal minor moderate major massive	low medium high	See comment above. • The potential future impact shall be assessed only for the risk assessment area.

2.16. 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?	minimal minor moderate major massive	low medium high	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
2.17. 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?	minimal minor moderate major massive	low medium high	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
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	minimal minor moderate major massive	low medium high	See below.
2.19. 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)?	minimal minor moderate major massive	low medium high	• 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".
2.20. 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?	minimal minor moderate major massive	low medium high	See above.
Economic impacts			
2.21. 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	minimal minor moderate major massive	low medium high	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.
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	minimal minor moderate major massive	low medium high	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.
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	minimal minor moderate major massive	low medium high	See above.
2.24. 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)?	minimal minor moderate major massive	low medium high	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".
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	minimal minor moderate major massive	low medium high	See above.
Social and human health impacts			
2.26. 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).	minimal minor moderate major massive	low medium high	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.
2.27. 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.	minimal minor moderate major massive	low medium high	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”.
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	minimal minor moderate major massive	low medium high	
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	NA minimal minor moderate major massive	low medium high	
2.30. 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?	minimal minor moderate major massive	low medium high	

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Entry	very unlikely unlikely moderately likely likely very likely	low medium high	
Summarise Establishment	very unlikely unlikely moderately likely likely very likely	low medium high	
Summarise Spread	very slowly slowly moderately rapidly very rapidly	low medium high	
Summarise Impact	minimal minor moderate major massive	low medium high	
Conclusion of the risk assessment	low moderate high	low medium high	

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)	Established (future)	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)	Established (future)	Invasive (currently)
Alpine				
Atlantic				
Black Sea				
Boreal				
Continental				
Mediterranean				
Pannonian				
Steppic				

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Established (future)	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				

ANNEXES

REFERENCES

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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
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 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.

[8] Not to be confused with „no impact".

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

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*

[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.

		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 strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*

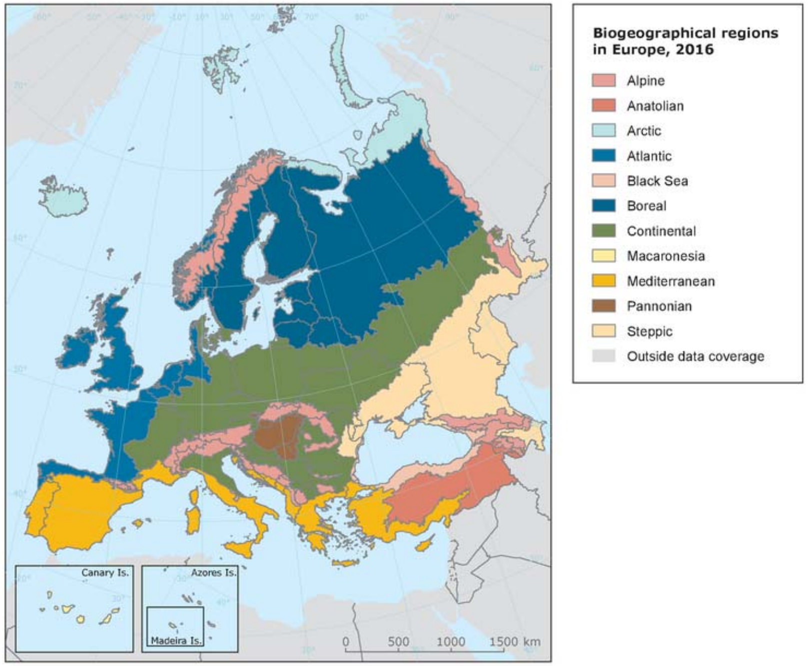
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. 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.*

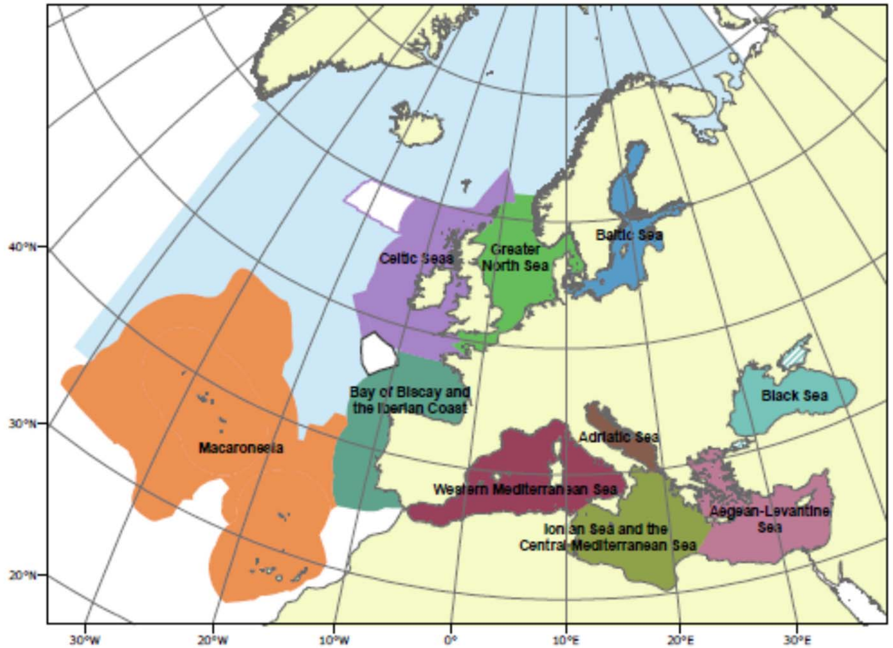
ANNEX V EU Biogeographic Regions and MSFD Subregions

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





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

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2[1]

Name of organism: *Koenigia polystachya* (Wall. ex Meisn.) T.M.Schust. & Reveal

Figure 1 *Koenigia polystachya* in Ireland (Image: Richard Shaw CABI)

[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

Author(s) of the assessment:
including the following elements:

- Rob Tanner, European and Mediterranean Plant Protection Organisation, Paris, France
- Etienne Branquart, Invasive Species Unit, Service Public de Wallonie, Gembloux, Belgium

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

Peer review 1: Oliver Pescott, NERC Centre for Ecology and Hydrology, Wallingford, UK
Peer review 2: Johan van Valkenburg, National Plant Protection Organization, Wageningen, Netherlands

This risk assessment has been peer-reviewed by two independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study.

Date of completion: 31/10/2018

Contents

RISK SUMMARIES

	RESPONSE	CONFIDENCE[2]	COMMENT
Summarise Entry[3]	**moderately likely**	**medium**	The entry pathway horticulture and transport (contaminant of soil) are the only relevant pathways for the entry of the species into the EU. However, a medium confidence has to be given as there is little evidence that the species is imported into the EU from outside of the risk assessment area.
Summarise Establishment[4]	**very likely**	**high**	The species is established within the risk assessment area in the following member states: Austria, Belgium, Czech Republic, Denmark, France, Germany, Ireland, Italy, Netherlands, Poland, United Kingdom. Further establishment is very likely.
Summarise Spread[5]	**moderately**	**medium**	In some Member States (UK for example), the species has shown rapid spread over a very short period of time (e.g. 2 years). Further spread is likely within the risk assessment area but a moderate rating of confidence is given as a rapid spread has not been realised in every member state where the species is established.
Summarise Impact[6]	**moderate**	**low**	Perennial knotweed species (*Fallopia*) in general are known to cause high impacts on the habitats they invade and include impacts on native biodiversity (plants and invertebrate populations). *K. polystachya* may have moderate impacts on biodiversity especially as it grows

[2] In a scale of low / medium / high, see Annex III
[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I
[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I
[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly
[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

			in more man-made habitats. In addition, the species may negatively impact on ecosystem services and have minimal socio-economic impact. However, there have been no specific scientific studies evaluating the impacts of *K. polystachya* and as a result a low level of confidence is given.
Conclusion of the risk assessment[7]	**moderate**	**medium**	An overall moderate score has been given for the risk assessment which accounts for the likeness of entry, the fact the species is established and the moderate spread potential of the plant. Impacts, although not scientifically evaluated, are likely to be moderate as the species can form dense monocultures which can outcompete native plant species in man-made habitats. However, with the lack of scientific studies a medium level of confidence is given.

[7] In a scale of low / moderate / high

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Austria	YES	YES	YES	
Belgium	YES	YES	YES	YES
Bulgaria	-	-	-	-
Croatia	-	-	-	-
Cyprus	-	-	-	-
Czech Republic	YES	YES	YES*	-
Denmark	YES	YES	YES	-
Estonia	-	-	YES*	-
Finland	-	-	YES	-
France	YES	YES	YES*	YES
Germany	YES	YES	YES*	-
Greece	-	-	-	-
Hungary	-	-	-	-
Ireland	YES	YES	YES	YES
Italy	YES	YES	YES*	-
Latvia	-	-	YES*	-
Lithuania	-	-	YES*	-
Luxembourg	-	-	YES	-
Malta	-	-	-	-
Netherlands	YES	YES	YES	-
Poland	YES	YES	YES*	-
Portugal	-	-	-	-
Romania	-	-	YES*	-

Slovakia	-	-	YES*	-
Slovenia	-	-	YES*	-
Spain		-	YES	-
Sweden	YES	-	YES	-
United Kingdom	YES	YES	YES	YES

* But to a much lower extent

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Alpine	YES	YES	YES	-
Atlantic	YES	YES	YES*	YES
Black Sea	-	-	-	-
Boreal	YES	YES	YES	-
Continental	YES	YES	YES*	-
Mediterranean	-	-	YES	-
Pannonian	-	-	-	-
Steppic	-	-	-	-

* But to a much lower extent

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	**Taxonomy:** Scientific name: *Koenigia polystachya* (Wall. ex Meisn.) T.M.Schust. & Reveal Kingdom: Plantae; Phylum: Magnoliophyta; Class: Angiospermae; Order: Caryophyllales; Family: Polygonaceae; Genus: *Koenigia* Note: The most recent taxonomic treatment places Himalayan knotweed in *Koenigia* (Schuster *et al*.., 2015). Many databases and publications use other synonyms. Note that *Persicaria wallichii* Greuter & Burdet is not mentioned as a synonym in Schuster *et al*.. (2015) but is given as the preferred name for *Polygonum polystachyum* Wall. ex Meisn. in The Plant List (2013). **Synonyms:** *Aconogonon polystachyum* (Wall. ex Meisn.) M. Král *Peutalis polystachya* (Wall. ex Meisn.) Raf. *Persicaria polystachya* (Wall. ex Meisn.) H. Gross 1913 *Persicaria wallichii* Greuter & Burdet *Polygonum polystachyum* Wall. ex Meisn. *Reynoutria polystachya* (Wall. ex Meisn.) Moldenke *Rubrivena polystachya* (Wall. ex Meisn.) M. Král **Common name:** English: Himalayan knotweed, bell-shaped knotweed, cultivated knotweed; garden smartweed; Kashmir plume; Danish: syren-pileurt; Finish: seljatatar;

	French: renouée à nombreux épis; German: vielähriger-Knöterich, Himalaya-Knöterich; Italian: poligono a spighe numerose; Dutch: Afghaanse duizendknoop; Norway: syrinslirekne; Russian: горец многоколосый **Description of the species:** *Koenigia polystachya* is a perennial herb growing up to 40-120 cm, rarely up to 180 cm. The stem is unarmed, ascending to erect and branched, usually reddish-brown, often flexuous above, smooth to densely pubescent. Leaves are lanceolate to elliptic-lanceolate, (7.5-) 9-22 (-27) × 2.8-7.8 cm, smooth to densely pubescent above, sparsely to densely below. The inflorescence a wide and spreading panicles are, 4-11 x 1-5.5 cm. Individual flowers are 3-5 mm long, usually creamy-white or sometimes pinkish in colour. Seeds are brown and small (2.1-2.5 mm long, and 1.3-1.8 mm wide). The flowers of *K. polystachya* are heterostylous (~~distyly~~distylous), usually with scattered, numerous reddish glands, slightly fragrant.
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 wild, in confinement or associated with a pathway of introduction]	In the horticultural trade within the risk assessment area plants traded as *Persicaria polymorpha* or *Polygonum polymorhum* are morphologically very similar. Another species that recently gained popularity is *Koenigia weyrichii*, and this can be likewise confused. Currently, there is no evidence that *P. polymorpha* nor *Koenigia weyrichii* are invasive within the risk assessment area. *K. polystachya* can also be confused with Alaska wild-rhubarb (*Koenigia alaskana* (Small) T.M.Schust. & Reveal), which is native to Alaska. *K. alaskana* has petioles that are 0.8-3.5 mm long, inflorescences that are 0-4 cm long, and green-white to white flowers (Flora of North America Editorial Committee, 2015).
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	A rapid risk assessment has been produced by the GB Non-native Species Secretariat. Great Britain Non-Native Species Secretariat (NNSS, 2015): http://www.nonnativespecies.org/index.cfm?pageid=143 The summary of this GB risk assessment is as follows: Entry risk: very likely, confidence: very high Establishment risk: very likely, confidence: very high Spread risk: intermediate, confidence high Impacts risk: major, confidence medium Conclusion risk: medium, confidence medium

	Other assessments include: • National Biodiversity Ireland (2013): medium risk of impact as an invasive weed (score 16) http://www.biodiversityireland.ie/wordpress/wp-content/uploads/Invasives_taggedMediumImpact_2013RA3.pdf • Alaska Natural Heritage Program (ANHP, 2011): Invasiveness Rank 80/100 http://accs.uaa.alaska.edu/files/invasive-species/Persicaria_wallichii_RANK_POPO5.pdf • Belgium Biodiversity Platform (2018): Prioritization leading to regulation: score 10/12 (List B). http://ias.biodiversity.be/species/show/85 • Switzerland: info flora (2012): The species is included on the Black List of plants in Switzerland https://www.infoflora.ch/fr/assets/content/documents/neophytes/inva_poly_pol_f.pdf • Brittany (France): (Quere and Geslin, 2016) Listed as a IA1 plant: (plants presently present in the territory considered to be invasively invasive within natural or semi-natural plant communities, and competing with native species or producing significant changes in composition, structure and / or ecosystem functioning • Czech Republic: Pergl *et al.*., (2016): Listed on the Grey List: Species with lower impact, but for which some level of management and regulation is desirable In California *K. polystachya* is classified as an noxious weed (B List), Massachusetts, Montana, Oregon it is classified as a B designated weed, and Washington it is classified as a Class B noxious weed) (USDA 2011). The authors are not aware of any other risk assessments for this species.
A4. Where is the organism native?	*Koenigia polystachya* is native to central and eastern Asia (DiTomaso and Healy 2007, eFloras 2008). The species is native to China (Sichuan, Xizang and Yunnan Province), Afghanistan, Bhutan, India, Kashmir, Myanmar, Nepal, Pakistan and southern Tibet) (CABI, 2018; Flora of China, 2018). As the common name suggests, *K. polystachya* is native to high altitude regions occurring in forests and valleys between 2200 and 4500 m above sea level. The species is also recorded in Korea (Hong and Mun, 2003).
A5. What is the global non-native distribution of the organism outside the risk assessment area?	*Koenigia polystachya* has been introduced to North America, Europe, and New Zealand (Hinds and Freeman 2005, Bartoszek *et al*.. 2006, Landcare Research 2011). This species is recorded in the following US States: Alaska, California, Massachusetts, Montana, Oregon, and Washington (USDA 2011). *Koenigia polystachya* has been reported as uncommon in California, except perhaps in North and Central coastline. In Washington, this species has been reported as spreading vigorously (Whatcom County, 2016).

	In Canada in the following Provinces: British Columbia and Nova Scotia,. *K. polystachya* has been documented from Ketchikan and Metlakatla in the Pacific Maritime ecogeographic region of Alaska (AKEPIC 2011). *Koenigia polystachya* is considered an emerging invasive species in the Vancouver region (British Colombia) by the Greater Vancouver Invasive Plant Council (2009). An emerging invasive is defined by them as: currently found in isolated, sparse populations but are rapidly expanding their range within the region.
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?	Recorded: Terrestrial biogeographic regions: • Alpine, Atlantic, Boreal, Continental Established: Terrestrial biogeographic regions: • Alpine, Atlantic, Boreal, Continental
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?	Current climate: Atlantic, Alpine, Boreal, Continental and Mediterranean. Future climate: Atlantic, Alpine, Boreal, Continental and Mediterranean. Increased and prolonged temperatures as a result of climate change (extending the growing season) will increase the growth of *K. polystachya* and increase the growth of the rhizome structures below ground increasing the potential invasiveness of the species. *K. polystachya* prefers average temperatures greater than 10 °C). Increased drought periods however, as a result of climate change will potentially limit the invasiveness of the species (*K. polystachya* prefers annual precipitation > 430 mm < 860 mm annually). For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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.	Recorded in the following Member States: Austria, Belgium, Czech Republic, Denmark, France, Germany, Ireland, Italy, Netherlands, Poland, Sweden, United Kingdom

	Established in the following Member States: Austria, Belgium, Czech Republic, Denmark, France, Germany, Ireland, Italy, Netherlands, Poland, United Kingdom Webb & Chater (1964) regard *K. polystachya* as established in central and north-western Europe (e.g. Great Britain, Denmark, The Netherlands, Germany, France and Austria). Originally introduced to Britain as an ornamental garden plant. First recorded in cultivation in Britain in 1900 and by 1917 had spread to the wild in North Devon. Usually found in abandoned gardens and areas where garden waste has been dumped, e.g. roadsides. By 1986 it had been recorded in 205 10km squares across The United Kingdom, increasing to 374 by 1999 and 608 by 2010 (NNSS, 2015). In Ireland the species is described by the national Biodiversity Data Centre (2013) being established and as having a scattered distribution but locally abundant in many places. Pergl *et al.* (2016) record the species as established in the Czech Republic. In Poland the species was first reported by Schube (1927) from Gluchelaz in the Silesian Region (Bartoszek *et al.*, 2006). In Belgium first record was in 1898 (Verloove, 2006) as a rather rare, locally naturalized garden escape (Conolly, 1977). In addition, it was first recorded in 1898 in Oostende. Subsequently, the species was collected in numerous locations throughout Belgium and is well-established in several places: locally abundantly naturalized in the Kempen (Mol, at least since 1974 and Rijkevorsel, since 1995). Sometimes very persistent and probably naturalized elsewhere (Mirwart, Wijnegem, Petite-Chapelle). Usually found on canal- or river banks, road verges, sometimes in wasteland or as a relic of cultivation near houses (Verloove, 2017). In Italy is considered a naturalized alien and invasive; However, still no particular threats to biodiversity have been shown (Galasso *et al.*, 2006) *Koenigia polystachya* is resident in Sweden (GBIF, 2015). **Non-EU States (outside of the risk assessment area) but worth mentioning** *Koenigia polystachya* is distributed throughout Switzerland (Info Flora. 2012). *Koenigia polystachya* is established in Norway at four known localities (Lid & Lid 2005).

A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	The information is given separately for current climate and under foreseeable climate change conditions: Current climate: Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Ireland, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom Future climate: Austria, Belgium, Czech Republic*, Denmark, Estonia*, Finland, France*, Germany*, Ireland, Italy*, Latvia*, Lithuania*, Luxembourg, Netherlands, Poland*, Romania*, Slovakia*, Slovenia*, Spain*, Sweden, United Kingdom * Risk reduced in future compared to current conditions. Increased and prolonged temperatures as a result of climate change (extending the growing season) will increase the growth of *K. polystachya* and increase the growth of the rhizome structures below ground increasing the potential invasiveness of the species. *K. polystachya* prefers average temperatures greater than 10 oC). Increased drought periods however, as a result of climate change will potentially limit the invasiveness of the species (*K. polystachya* prefers annual precipitation > 430 mm < 860 mm annually). For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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?	Yes. In its native range, in India in the Valley of the Flowers National Park, dense monocultures are found in habitats affected by past anthropogenic pressures or natural disturbances such as eroded, avalanche-prone, rocky areas with a fragmented treeline. Most recently dense populations were also observed in various natural nutrient poor alpine and sub-alpine ecosystems (Kala and Shrivastava, 2004: Negi *et al* 2017). In Asia, it is considered an alien invasive plant in Sri Lanka, where it is reported to colonise riparian, wetlands, water streams and canals in Nuwara Eliya (central Sri Lanka) and surrounding areas (Gunasekera, 2016). *Koenigia polystachya* is invasive in North America. *Koenigia polystachya* is considered an emerging

	invasive species in the Vancouver region (Canada) by Greater Vancouver (Greater Vancouver Invasive Plant Council, 2009). In the United States, *Koenigia polystachya* has been documented from Ketchikan and Metlakatla in the Pacific Maritime ecogeographic region of Alaska (AKEPIC 2011). In Alaska the species can negatively impact native plant species (the edible species salmonberry *Rubus spectabilis* and thimbleberry *Rubus parviflorus*). CABI (2018) list the species as invasive in California, Montana, Oregon, and Washington (citing USDA-NRCS, 2015). In Washington, this species has been reported as spreading vigorously (NatureServe, 2015).
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	Terrestrial biogeographic regions: • Alpine, Atlantic, Continental, (InfoFlora 2012, NNSS 2015, Pergl *et al* 2016; Quere and Geslin, 2016)
A12. In which EU member states has the species shown signs of invasiveness?	Belgium, France, Ireland, United Kingdom (including Scotland) The Belgium Biodiversity Platform (2018) state '*P. wallichii* [*K. polystachya*] grows vigorously and creates large, dense and persistent colonies that exclude native vegetation and prevents the establishment of tree seedlings. It also favours erosion of river banks and greatly alter natural ecosystems'. In Ireland, *K. polystachya* can form monocultures along road sides (Follak *et al*.., 2018) which can over shadow and outcompete native plant species (Personal observation, Tanner, 2009). According to Hill *et al*.. (2009), the adverse impacts of *P. wallichii* [*K. polystachya*] on native British species in terms of competition carries a 'high risk'. It can cause (> 80%) population declines of valued or rare species, and may reduce local species richness irreversibly. At a regional scale, it may cause species decline. However, Hill et al. (2009) also highlights that in the UK poses a 'medium risk' to natural and semi-natural habitats, and may occasionally colonize these areas. In France the species has shown invasive behaviour (Quere and Geslin, 2016). As such the species is listed as a IA1 plant: (plants presently present in the territory considered to be invasive within natural or semi-natural plant communities, and competing with native species or producing significant changes in composition, structure and / or ecosystem functioning).
A13. Describe any known socio-economic benefits	Apart from the value of the species as an ornamental plant sold by the horticulture trade,

of the organism.	*Koenigia polystachya* has little socio-economic benefits to the risk assessment area. The species is available in the horticultural trade as an ornamental garden plant and is often regarded as easy to grow with fragrant flowers. The species is available for sale from 7 suppliers recommended by the RHS plant finder (https://www.rhs.org.uk/Plants/Search-Results?formmode=true&context=l%3Den%26q%3DPersicaria%2Bwallichii%26sl%3DplantForm&query=Persicaria%20wa llichii). Outside of the risk assessment area, the plant is utilised as a vegetable in India (CABI, 2018) and Tibet (Boesi, 2014) but there is no evidence that the species is utilised for this purpose in the risk assessment area.

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."
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	few	high	The only pathways relevant for the entry of the species into the risk assessment area is via the horticulture trade - horticulture (escape from confinement) and transport – Contaminant (transport of habitat material (soil, vegetation).

[8] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf
[9] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	**(1) Horticulture (escape from confinement).** **(2) transport – Contaminant (transport of habitat material (soil, vegetation)**		The main pathway for this species is introduction via the horticulture trade as plants for planting. Historically this is how the species entered the risk assessment area (see Belgium Biodiversity Forum, 2007 and Ison 2011).
Pathway name:	**(1) Horticulture (escape from confinement).**		
1.3. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional	high	Entry via horticulture is an intentional pathway.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	**moderately likely**	**medium**	Although this pathway has been detailed as a historic pathway for the entry of the species into the risk assessment area (see Branquart *et al.*., 2007 and Ison 2011), there is no evidence that large volumes of the species are imported into the risk assessment area, probably due to the species not being imported from outside of the EU and it appears to have been replaced in trade by *P. polymorpha* and *K. weyrichii*. To highlight this point, an internet search for suppliers from ebay and amazon produced no results. Plantlife (2010) also note that the species is less popular as an ornamental species in recent years. Therefore, it is only moderately likely that large numbers of the organism will travel along this pathway. Information on volumes is not available.

			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.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**very likely**	**high**	The pathway ‘Horticulture (escape from confinement)’ 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. It is unlikely that *K. polystachya* will multiply along the pathway - Horticulture (escape from confinement) during transport and storage. Rhizomes would be the most likely plant parts for transport, rather than whole plant parts or seeds. Rhizome structures are robust and when packed appropriately could survive prolonged transport. However, cuttings and bare rooted plants or potted plants may also be used.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	**very likely**	**high**	The pathway ‘Horticulture (escape from confinement)’ 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.
1.7. How likely is the organism to enter the risk assessment area undetected?	**unlikely**	**medium**	It is unlikely that the organism will enter the risk assessment area undetected as the pathway ‘Horticulture (escape from confinement)’ is the deliberate movement of plant material into the risk assessment area.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**very likely**	**high**	It is very likely that the organism will arrive during the months of the year most appropriate for establishment as the pathway ‘Horticulture (escape from

			confinement)' is the deliberate movement of plant material into the risk assessment area. This can occur all year round.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**very likely**	**high**	As the pathway is horticulture, which would result in the deliberate planting of the species in an outdoors situation, it is very likely that the species can transfer from this pathway to a suitable habitat.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**moderately likely**	**medium**	A likely score has been given for the overall entry into the risk assessment area as the species has been recorded as entry via this pathway historically. However, the likely score as opposed to very likely coupled with the medium uncertainty is given as there is no evidence that the species enters the risk assessment area via this pathway in current times.
Pathway name:	**(2) Transport – Contaminant (transport of habitat material (soil, vegetation)**		
1.3. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	unintentional	high	Entry via movement of soil or vegetation (Soll, 2004).
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	**moderately likely**	**medium**	The transport of top soil and or other contaminated material with rhizomes of the species can facilitate entry into the RA area. There is the potential for numerous rhizomes to be transported along this pathway and only a small amount of rhizome is needed to produce a viable plant.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)?	**very likely**	**high**	The pathway Transport – Contaminant (transport of habitat material (soil, vegetation) is the unintentional movement of plant material into the risk assessment

Subnote: In your comment consider whether the organism could multiply along the pathway.			area. As the rhizomes would be moved with soil it is likely that they would survive during passage. It is unlikely that *K. polystachya* will multiply along the pathway Rhizomes would be the most likely plant parts for transport, rather than whole plant parts or seeds. Rhizome structures are robust and when packed appropriately could survive prolonged transport.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	**likely**	**high**	Soil is unlikely to be treated as it is moved through the pathway and as such plant material would survive.
1.7. How likely is the organism to enter the risk assessment area undetected?	**likely**	**high**	It is likely that the organism will enter the risk assessment area undetected as rhizome material will be hidden in soil and only a small rhizome is needed to produce a viable plant.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**very likely**	**high**	It is very likely that the organism will arrive during the months of the year most appropriate for establishment as movement on this pathway can occur all year round.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**very likely**	**high**	As the pathway involves the movement of soil this may result in the deliberate positioning of soil (which could be contaminated with rhizome material) in an outdoors situation, it is very likely that the species can transfer from this pathway to a suitable habitat.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**moderately likely**	**medium**	A moderately likely score has been given for the overall entry into the risk assessment area. However, the likely score as oppose to very likely coupled with the medium uncertainty is given as there is no evidence that the species enters the risk assessment area via this pathway in current times.

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. If the species is established in all Member States, continue with Question 1.16.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	**very likely**	**high**	It is very likely that *Koenigia polystachya* will be able to establish in the risk assessment area with a high level of confidence. The species is already established within the risk assessment area (Austria, Belgium, Czech Republic, Denmark, France, Germany, Ireland, Italy, Netherlands, Poland, United Kingdom). Climatic conditions in the EU, particularly in the Atlantic and Continental regions, are similar to those found in the aforementioned countries where the species has formed established populations. In addition, the species could become established in the Alpine and Boreal biogeographical regions.
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	**very likely**	**high**	*K. polystachya* has a wide tolerance to soil conditions begin able to grow in soils seasonally waterlogged to free draining soils. *K. polystachya* grows best in nutrient-rich soils (FOEN, 2006; Alaska Natural Heritage Program, 2011). The species is already established within the risk assessment area (Austria, Belgium, Czech Republic, Denmark, France, Germany, Ireland,

			Italy, Netherlands, Poland, United Kingdom) further establishment is very likely.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	**widespread**	**high**	The habitats necessary for the survival of the species are widespread within the RA area. *K. polystachya* grows best in unshaded areas (WSDA 2008) and seedlings may not survive in shaded areas. This species grows in moist, disturbed sites, roadsides, fields, and waste areas (Hinds and Freeman 2005, DiTomaso and Healy 2010, Klinkenberg 2012). In Poland, it has established only in anthropogenically disturbed areas (Bartoszek 2006). However, it can also establish in areas disturbed by river action or flooding (Washington State Noxious Weed Control Board, 2004). The species grows along riverbanks in the risk assessment area. In Ireland, linear monocultures occur alongside roadsides (personal observation, Tanner).
1.16. 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 ?	**NA**	**high**	*K. polystachya* does not require another species for any part of its lifecycle.
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	**very likely**	**high**	It is very likely that *K. polystachya* will establishment despite competition from existing species. *K. polystachya* is highly competitive species which grows from an underground rhizome network established in previous seasons. The species emerges early in the growing season (before many native species) and can grow up to 2 metres in height which act to outshade native vegetation (DiTomaso and Healy 2007, Wilson, 2007). The species can form dense monocultures

			which exclude native plants species.
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	**very likely**	**high**	There are no host specific natural enemies within the risk assessment area. Any generalist organisms which feed on or infect *K. polystachya* will not prevent its establishment.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	**moderately likely**	**high**	There are a number of management practices applied to 'knotweed' species within the risk assessment area and those management practices for *Fallopia japonica* can be applied for *K. polystachya*. However, these management practices are mainly applied to established populations and not to prevent establishment.
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	**likely**	**high**	The establishment of *K. polystachya* is suited to disturbed habitats especially along roadsides and disused waste ground. It is therefore likely that the current urbanization trend occurring in Europe may favor the establishment of the species.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	**likely**	**high**	The extensive creeping rhizome underground network produced by the species makes eradication problematic as all underground plant material will need to be eradicated. Root and stem fragments as small as 1cm in length can form new plants colonies (Soll, 2004; NNSS, 2015).
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	**very likely**	**high**	*K. polystachya* is a perennial that reproduces sexually by seed and vegetatively by rhizomes and stem fragments (Soll, 2004; NNSS, 2015). The requirements for seed germination are not documented within the risk assessment area and it is unclear if the seeds are a major component of establishment of the species. Ison (2011) report that seed production is rare in the UK. However, similar to other knotweed species, disturbance (and rhizomes within the soil) can promote the

			establishment of the species. A rhizome fragment as small as 1 cm in length can produce a viable plant.
1.23. How likely is the adaptability of the organism to facilitate its establishment?	**very likely**	**high**	The species is very adaptable, and this is shown with the wide range of habitats and abiotic conditions within which the species can grow. It should also be highlighted that in the plants native range the species grows at high altitude elevations whereas in the risk assessment area, the species can establish at significantly lower elevations.
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	**very likely**	**high**	As previously highlighted, seed production and seed germination are not considered a major reproductive component for the plant. Therefore, as the species multiplies by rhizomes – this will result in a lower genetic diversity. This is not likely to prevent the species from establishing.
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	**very likely**	**high**	*Koenigia polystachya* has been introduced to North America, Europe, and New Zealand (Hinds and Freeman 2005, Bartoszek *et al.*. 2006, Landcare Research 2011). This species is recorded in the following US States: Alaska, California (classified as an noxious weed B List), Massachusetts, Montana, Oregon (B designated weed), and Washington (classified as a Class B noxious weed) (USDA 2011) and in Canada in the following Provinces: British Columbia and Nova Scotia,. *K. polystachya* has been documented from Ketchikan and Metlakatla in the Pacific Maritime ecogeographic region of Alaska (AKEPIC 2011). *Koenigia polystachya* is considered an emerging invasive species in the Vancouver region (British

			Colombia) by the Greater Vancouver Invasive Plant Council (2009). An emerging invasive is defined by them as: currently found in isolated, sparse populations but are rapidly expanding their range within the region. The species is already established within the risk assessment area (Austria, Belgium, Czech Republic, Denmark, France, Germany, Ireland, Italy, Netherlands, Poland, United Kingdom) and further establishment is highly likely.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	**very likely**	**high**	The species is already established within the risk assessment area.
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	**very likely**	**high**	Atlantic, Alpine, Boreal and Continental
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	**very likely**	**high**	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 (2070)~~ • ~~the applied scenario (eRCP 4.5)~~ Increased and prolonged temperatures as a result of climate change (extending the growing season)

			will increase the growth of *K. polystachya* and increase the growth of the rhizome structures below ground increasing the potential invasiveness of the species. *K. polystachya* prefers average temperatures greater than 10 °C). Increased drought periods however, as a result of climate change will potentially limit the invasiveness of the species (*K. polystachya* prefers annual precipitation > 430 mm < 860 mm annually). Modelling by the Centre of Ecology and Hydrology (annex VI) suggests there will be a significant decrease in suitability within Atlantic, Black Sea, Continental and Mediterranean regions. However, there will be an increase in the alpine and boreal Arctic biogeographical region.

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	**moderate**	**high**	In general, knotweed rhizomes and stem pieces are transported along waterways and by flooding (DiTomaso and Healy, 2007). Knotweeds can also be dispersed short distances in sea water (Wilson, 2007). Knotweeds can regenerate from <2 cm rhizome (Wilson, 2007). NNSS (2015) notes that seed production is rare and some populations appear to be sterile in the PRA area. Requirements for seed germination/viability are unknown (CABI, 2017). However, others note that *K. polystachya* flowers are perfect (bisexual) and plants regularly produce seed (Wilson, 2007). The small seeds are dispersed by wind/water. Seed production has been reported to be low in California, British Columbia (Alaska Natural Heritage Program (2011). A moderate rating has been given for spread as in some countries where the species is present (e.g. AT, BE and CZ) distribution trends do not show a rapid spread.
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance?	**major**	**medium**	*K. polystachya* is planted as an ornamental in gardens in the EPPO region. In the UK, there are 7 suppliers

(Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.			in the RHS Plant Finder (https://www.rhs.org.uk/). This species has been promoted by the Daily Telegraph in the UK: http://www.telegraph.co.uk/gardening/plants/10634486/Top-10-plants-for-a-rainy-day.html?frame=2820359 *K. polystachya* has escaped cultivation (CABI, 2017). Dumped garden waste may contain rhizomes and stem fragments (NNSS, 2015). The species can be spread by soil (as a contaminant) especially as only small amounts of rhizomes can form viable plants (Soll, 2004). The one country with a long history of cultivation of *K. polystachya* (UK) has recorded high rates of spread (NNSS, 2015).
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	UNAIDED (natural dispersal) Transport – Contaminant (transport of habitat material (soil, vegetation)		
Pathway name:	**UNAIDED (natural dispersal)**		
2.3. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	**unintentional**	**high**	
2.4. 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?	**moderately likely**	**high**	One root fragment as small as 1 cm in length can form new plant colonies (CABI, 2018).

2.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**very likely**	**high**	Although there is no research conducted on specific aspects of regeneration in rhizomes for *K. polystachya*, there has been research conducted on other knotweed species. A high rhizome regeneration for *Fallopia japonica* var. *japonica* has been recorded for both terrestrial and aquatic environments highlighting that knotweeds can persist in water bodies for prolonged periods of time and be carried through waterbodies.
2.6. How likely is the organism to survive existing management practices during spread?	**very likely**	**medium**	As 1 cm of rhizome in length can form new plant colonies management practices would need to exhaust all underground plant material which is often impractical along waterbodies.
2.7. How likely is the organism to spread in the risk assessment area undetected?	**very likely**	**high**	As 1 cm of rhizome in length can form new plant colonies, small fragments can be incorporated into waterbodies and spread through the risk assessment area undetected.
2.8. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	**very likely**	**high**	If spreading through a riparian system the species is very likely to transfer to a suitable habitat.
2.9. Estimate the potential rate of spread within the Union based on this pathway (please provide quantitative data where possible)	**moderately**	**medium**	In the UK the species has been shown to spread rapidly (however, not due to natural dispersal) (NNSS, 2015), however, it is not clear and unlikely to be due to natural spread. A moderate score has been given as the species has not shown similar high spread in other EU Member States (Branquart pers comm., 2018).
End of pathway assessment, repeat as necessary.			
Pathway name:	**Transport – Contaminant (transport of habitat material (soil, vegetation)**		The transport of top soil and or other contaminated material with rhizomes of the species can facilitate spread within the RA area.
2.3. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional	**unintentional**	**high**	The species would be spread through the contaminant of top soil or other material and thus it is an

(the organism is a contaminant of imported goods)?			unintentional pathway of spread.
2.4. 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?	**very likely**	**high**	One root fragment as small as 1 cm in length can form new plant colonies (CABI, 2018).
2.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**very likely**	**high**	Although there is no research conducted on specific aspects of regeneration in rhizomes for *K. polystachya*, there has been research conducted on other knotweed species. For *Fallopia japonica* var. *japonica*, as little as 0.7g of root material is sufficient to establish new plants (Brock and Wade, 1992).
2.6. How likely is the organism to survive existing management practices during spread?	**likely**	**medium**	Careful methodical management practices would be needed to ensure that the species did not spread with contaminated soil. This is often not feasible with such small rhizomes.
2.7. How likely is the organism to spread in the risk assessment area undetected?	**very likely**	**high**	Small amounts of rhizomes can regenerate into large plants and thus they can remain buried in top-soil undetected.
2.8. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	**very likely**	**high**	Top soil would be physically transferred to suitable habitats and thus it is very likely that the species will transfer to suitable habitats.
2.9. Estimate the overall potential for spread within the Union based on this pathway?	**moderately**	**high**	Although there is no evidence of the movement of the species along this spread pathway, it could be a rapid movement – a low confidence score highlights the lack of information.
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	**with some difficulty**	**medium**	The species can spread via natural dispersal which will, will some difficulty be able to be prevented due mainly to connecting water bodies. In addition, spread by contamination will be difficult to prevent as the rhizomes which can regenerate into a viable plant are small.
2.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions	**moderately**	**low**	Within the Atlantic, Black Sea, Continental and Mediterranean regions there is a moderate potential

for this organism in the risk assessment area (using the comment box to indicate any key issues and please provide quantitative data where possible).			for spread.
2.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (please provide quantitative data where possible)	**moderately**	**low**	Within the Atlantic, Black Sea, Continental and Mediterranean regions there is a moderate potential for spread.

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is 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?	**major**	**medium**	Dense foliage restricts light to other plants (Info Flora, 2013). *K. polystachya* pushes back [outcompetes] native bushes of edible salmonberry and thimbleberry (eaten fresh and preserved in Alaska) (see http://www.uaf.edu/files/ces/cnipm/annualinvasivespeciesconference/13thAnnualMeetingProceedings/Winter%20-%20Economic%20impacts%20CNIPM%20Presentation%202012%20.pdf) It also grows very quickly and outcompetes native plant species in Pacific Northwest, USA (Natureserve Explorer, 2015) [Himalayan knotweed impacts riparian areas (Skamania County, Washington, Noxious Weeds; WA State Noxious Weed Control Board 2003). It is known to exclude native species (Skamania County, Washington, Noxious Weeds).]

			Many relatives of *K. polystachya* are major invasive species for which more documentation on impacts exists e.g. *Fallopia japonica, F. sachalinensis and F. x bohemica* Lavoie 2017). The commonly cited WSDA (2008) actually addresses 4 knotweeds together and is not specific to Himalayan knotweed. A negative impact of knotweeds (generally) on invertebrates (i.e. reduced abundance and species richness) is mentioned in WSDA (2008) and demonstrated by a European study of *F. japonica, F. sacchalinensis* and *F. x bohemica* by Gerber *et al.*. (2008). There is no data specifically for the impact of *K. polystachya* on invertebrates and higher levels of the food chain. *K. polystachya* has large leaves and produces thick foliage, which outshades underlying vegetation (WSDA 2008) and displaces native species (DiTomaso and Healy 2007). This species can limit the establishment of trees (WSDA 2008). *K. polystachya* can reduce the quality of fish and wildlife habitat in riparian areas. Infestations may reduce insect populations that provide food sources to salmon (WSDA 2008).
2.14. 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)?	**moderate**	**low**	To-date there are no know studies that have scientifically evaluated the impact of *K. polystachya* in the risk assessment area. According to Hill *et al.*. (2009), the adverse impacts of *K. polystachya* on native British species in terms of competition carries a ‘high risk’. It can cause local severe (> 80%) population declines of valued or rare species, and may reduce local species richness irreversibly. At a regional scale, it may cause species

			decline. Impacts, although not scientifically evaluated, are likely to be moderate as the species can form dense monocultures which can outcompete native plant species but the current populations within the EU are mainly within man-made habitats (such as along roads) although some of them may be found also in riparian ecosystems (Hill *et al*., 2009; NNSS, 2015; Gunasekera, 2016; Floron 2018). However, with the lack of scientific studies a low level of confidence is given.
2.15. 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?	**moderate**	**medium**	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 but this occurs mainly in man-made habitats.
2.16. 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?	**moderate**	**low**	At present within the risk assessment area there have been no studies conducted on the impact of *K. polystachya* on native plant species. According to Hill *et al*.. (2009), *K. polystachya* in the UK poses a 'medium risk' to natural and semi-natural habitats, and may occasionally colonize these areas. However, populations of this species are usually confined to habitats with low or medium conservation value. *K. polystachya* also brings a 'medium risk' of altering ecosystem function, including nutrient cycling, physical alteration, successions and food webs.
2.17. 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?	**moderate**	**medium**	As a species that has the tendencies to form monospecific stands, there is the potential of the species having a high impact on native biodiversity but as Hill *et al*.., 2009 details the species normally colonises habitats with a low or medium conservation value. In Poland the species 'occurs exclusively in habitats evidently suffering more or less from human impact,

			where it is accompanied by ubiquitous native and synanthropic species' (Bartoszek *et al*.., 2006).
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	**moderate**	**medium**	No specific scientific studies have been conducted on the impacts of *K. polystachya* on ecosystem services and thus all information comes from observations. It is documented that in the USA, *K. polystachya* reduces the availability of nutrients in the soil. It competes with trees and can reduce shade along rivers and streams by displacing native, woody species (WSDA 2008). Infestations produce dense mats of leaf litter that prevent the germination of native species (Wilson 2007).
2.19. 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)?	**moderate**	**medium**	No specific scientific studies have been conducted on the impacts of *K. polystachya* on ecosystem services and thus all information comes from observations. As a species that can grow in riparian systems, *K. polystachya* has the potential of negatively impacting on cultural ecosystem services by reducing access to water bodies for recreational activities. The species can also invade urban areas of cultural importance thereby decreasing the appeal. Hill *et al*.., (2009) suggests the impact on ecosystem processes and structures is moderate and reversible.
2.20. 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?	**moderate**	**medium**	See above comments in question 2.19. With increased spread and established populations, *K. polystachya* will potentially have moderate impacts within Atlantic, Alpine, Boreal, Continental biogeographical regions in the future.
Economic impacts			
2.21. How great is the overall economic cost caused by the organism within its current area of distribution	**moderate**	**medium**	There are no known economic assessments of *K. polystachya* in the current area of distribution excluding

(excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management			the risk assessment area. Control costs for knotweed species can be high and involve significant resources and labour-intensive methods including removal of contaminated soils, however there are no figures available for the species. Kala (2004) suggests that the species can reduce the value of pasture land in the plants native range though no monetary figures are given. Control costs for knotweed species can be high and involve significant resources and labour-intensive methods including removal of contaminated soils, however there are no figures available for the species. In Washington State, USA, when invasive knotweeds are taken together (*Fallopia sachalinense*, *K. polystachya*, *Fallopia japonica and Fallopia bohemica*) the annual direct economic impact per county is estimated at $48 000.
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	**minor**	**medium**	The species can have negative implications for home sellers and buyers as the presence of the species can prevent banks from lending money http://www.telegraph.co.uk/finance/personalfinance/borrowing/mortgages/12012333/Now-its-not-only-knotweed-that-will-stop-you-getting-a-mortgage.html
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	**minor**	**medium**	See above.
2.24. 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)?	**minor**	**low**	No information has been found on the issue.

2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	**minor**	**low**	See above.
Social and human health impacts			
2.26. 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).	**minor**	**medium**	The species can have negative implications for home sellers and buyers as the presence of the species can prevent banks from lending money http://www.telegraph.co.uk/finance/personalfinance/borrowing/mortgages/12012333/Now-its-not-only-knotweed-that-will-stop-you-getting-a-mortgage.html. There are no known human health impacts known for this species.
2.27. 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.	**moderate**	**low**	No information has been found on the issue
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	**minimal**	**high**	There are no host specific natural enemies within the risk assessment area feeding on the species.
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	**NA**	**medium**	
2.30. 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?	**moderate**	**medium**	NA: there are no natural enemies within the risk assessment area.

ANNEXES

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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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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 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.

[10] Not to be confused with „no impact“.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.*. 2017)

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[11]	**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.*

[11] 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.

	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** 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*

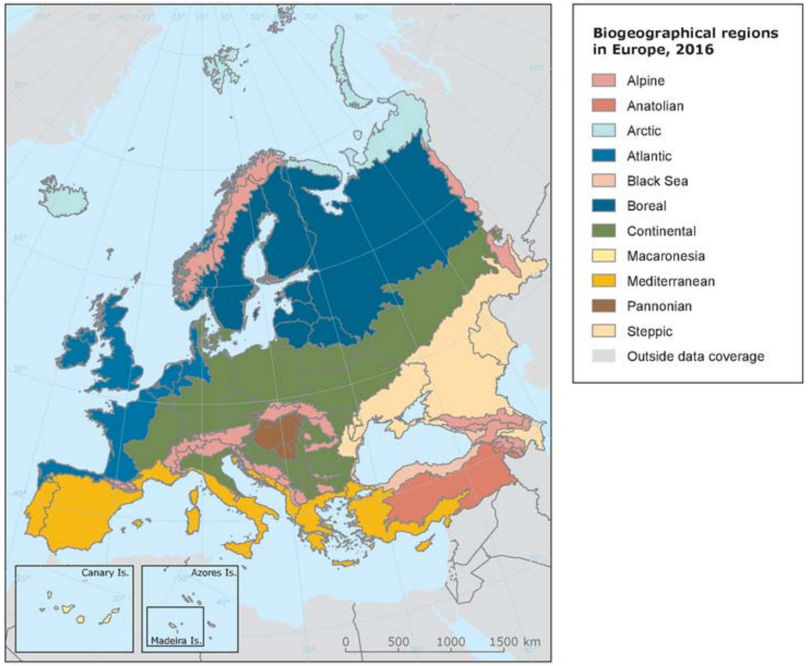
			composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living 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.*

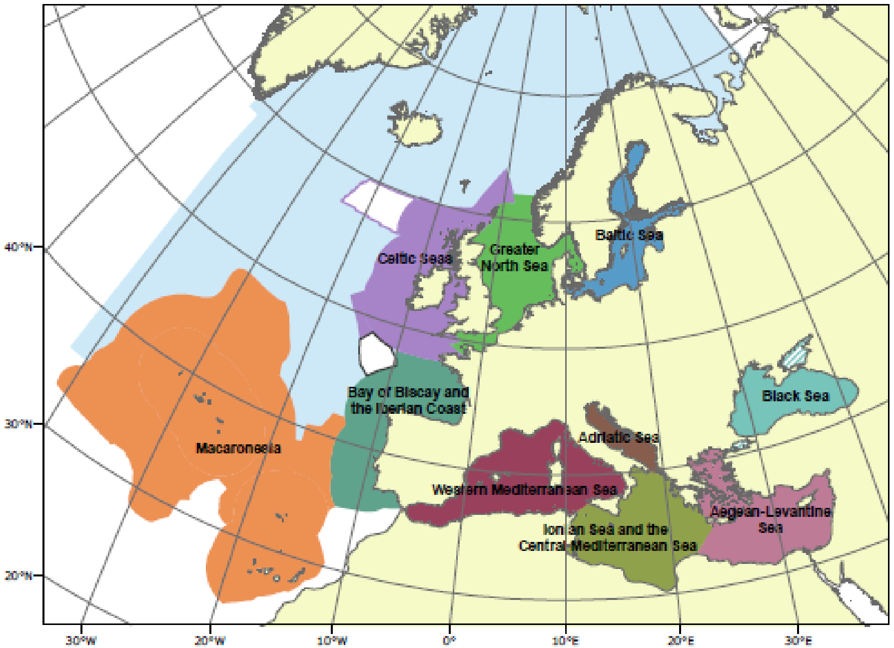
ANNEX V EU Biogeographic Regions and MSFD Subregions

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: Projection of climatic suitability for *Koenigia polystachya* establishment

Daniel Chapman
20th July 2018

Aim
To project the climatic suitability for potential establishment of *Koenigia polystachya* in Europe, under current and predicted future climatic conditions.

Data for modelling
Species occurrence data were obtained by searching multiple large online databases for all synonyms of *Koenigia polystachya* listed by the Global Biodiversity Information Facility (GBIF). The data sources searched were GBIF, Early Detection and Tracking System (EDDMaps), Atlas of Living Australia (ALA), USGS Biodiversity Information Serving Our Nation (BISON), Berkeley Ecoinformatics Engine, Integrated Digitized Biocollections (iDigBio) and iNaturalist, as well as a personal database of native range records (Rob Tanner, *pers. comm.)*.
We scrutinised occurrence records from regions where the species is not known to be established and removed any that appeared to be dubious 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 (Figure 1a). This resulted in a total of 533 grid cells containing records of *K. polystachya* for the modelling (Figure 1a), which is a reasonable number for distribution modelling.
Current day climate data representing 1960-1990 average conditions were taken from the bioclimatic variables contained within the WorldClim v1 database (Hijmans et al., 2005). These were originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and were aggregated to a 0.25 x 0.25 degree grid for use in the model. Consideration of the likely limiting factors on establishment by *Koenigia polystachya* in Europe led to selection of the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6 °C) reflecting winter cold stress.
- Mean temperature of the warmest quarter (Bio10 °C) reflecting the summer thermal regime.
- Climatic moisture index (CMI, ratio of mean annual precipitation, Bio12, to annual potential evapotranspiration, PET) reflecting plant moisture regimes. To calculate CMI, monthly PETs 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). *Koenigia polystachya* occurs in relatively humid environments and might be restricted by excessive drought stress. CMI was log+1 transformed for analysis.
- Precipitation seasonality (Bio15, the coefficient of variation among monthly precipitations), reflecting the likelihood of periodic drought or waterlogging stress.

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathway (RCP) 4.5 and 8.5 were also obtained. For both scenarios, 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).

RCP 4.5 is a moderate climate change scenario in which CO_2 concentrations increase to approximately 575 ppm by the 2070s and then stabilise, resulting in a modelled global temperature rise of 1.8 °C by 2100 (90^{th} percentile range 1.1-2.6 °C) (IPCC Working Group I, 2013). RCP8.5 is the most extreme of the RCP scenarios, and may therefore represent the worst case scenario for reasonably anticipated climate change. In RCP8.5 atmospheric CO_2 concentrations increase to approximately 850 ppm by the 2070s, resulting in a modelled global mean temperature rise of 3.7 °C by 2100 (90^{th} percentile range 2.6 to 4.8°C) (IPCC Working Group I, 2013).

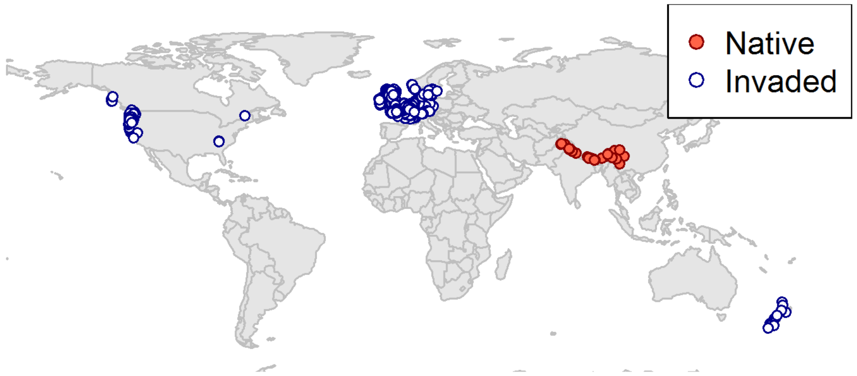
The model also included one non-climatic predictor to capture a possible association between human activities and invasive non-native species:

- Human influence index from the Global Human Influence Index Dataset of the Last of the Wild Project (WCS & CIESIN, 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 log+1 transformed for the modelling to improve normality.

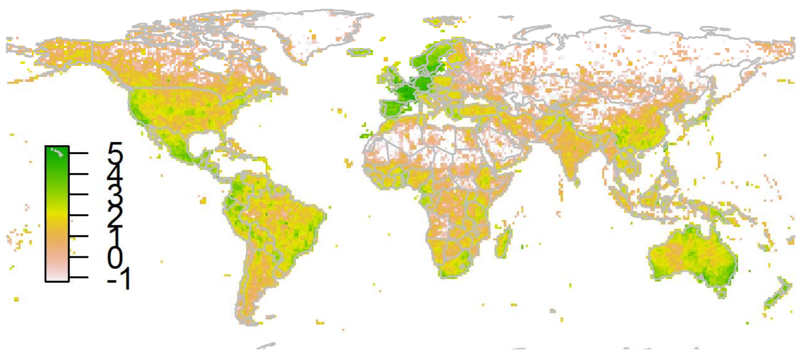
Finally, the recording density of vascular plants (phylum Tracheophyta) on GBIF was obtained as a proxy for spatial recording effort bias (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Koenigia polystachya* and used in the modelling, showing the native range and (b) a proxy for recording effort – the number of vascular plant records (phylum Tracheophyta) held by the Global Biodiversity Information Facility, displayed on a log_{10} scale.

(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 Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale (Elith et al., 2010), we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to. Therefore background samples (pseudo-absences) were sampled from two distinct regions:

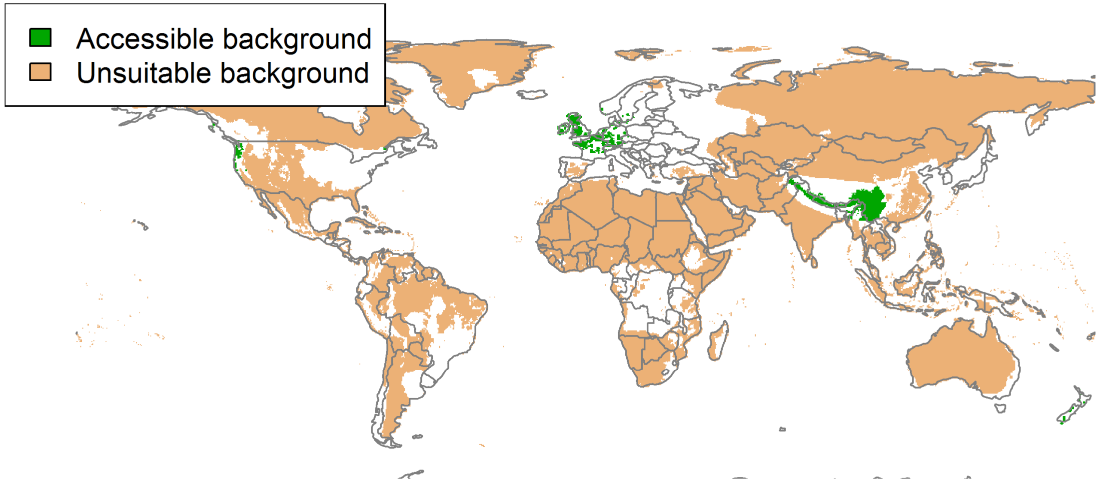
- An accessible background includes places close to *K. polystachya* populations, in which the species is likely to have had sufficient time to disperse and sample the range of environments. We defined the accessible background as a 400 km buffer around the minimum convex polygon bounding native records and a 40 km buffer around non-native records. Accessibility was more restricted in the invaded range to account for stronger dispersal constraint over a shorter residence time, as well as reports of greater reliance on vegetative reproduction in the invaded range (CABI, 2018), which may be less dispersive. Prior testing of the model methods shows the choice of buffer distance is usually not critical to the modelling.

- An unsuitable background includes places with an expectation of environmental unsuitability, e.g. places too cold or dry. Absence from these regions should be irrespective of dispersal constraints, allowing inclusion of this background in the modelling. No specific ecophysiological information was available to define the unsuitable region, but based on expert opinion that temperature and drought are likely to be limits on *K. polystachya* occurrence in Europe unsuitability was defined as:
  - Minimum temperature of the coldest month (Bio6) < -20 °C, OR
  - Mean temperature of the warmest quarter (Bio10) < 4 °C, OR
  - Mean temperature of the warmest quarter (Bio10) > 26 °C, OR
  - Climatic moisture index (CMI) < 0.45.

None of the occurrences fell within the unsuitable background.
Ten random background samples were obtained:

- From the accessible background 533 samples were drawn, which is the same number as the occurrences. Sampling was performed with similar recording bias as the distribution data using the target group approach (Phillips, 2009). In this, sampling of background grid cells was weighted in proportion to GBIF recording density (Figure 1b). Taking the same number of background samples as occurrences ensured the background sample had the same level of bias as the data.
- From the unsuitable background 3000 simple random samples were taken. Sampling was not adjusted for recording biases as we are confident of absence from these regions. Model testing on other datasets has shown that this method is not overly sensitive the number of unsuitable background samples.

Figure 2. The background regions from which 'pseudo-absences' were sampled for modelling. The accessible background is assumed to represent the range of environments the species has had chance to sample. The unsuitable background is assumed to be environmentally unsuitable for the species.



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 (except where specified below) and rescaled using logistic regression:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per effect.
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent (Phillips et al., 2008)

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 calculating the Area Under the Receiver-Operator Curve (AUC) for model predictions on the evaluation data, which were reserved from model fitting. AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected pseudo-absence.

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.

Global model projections were made for the current climate and for the two climate change scenarios, avoiding model extrapolation beyond the ranges of the input variables. The optimal threshold for partitioning the ensemble predictions into suitable and unsuitable regions was determined using the 'minimum ROC distance' method. This finds the threshold where the Receiver-Operator Curve (ROC) is closest to its top left corner, i.e. the point where the false positive rate (one minus specificity) is zero and true positive rate (sensitivity) is one.

Limiting factor maps were produced following Elith et al. (2010). 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. Partial response plots were also produced by predicting suitability across the range of each predictor, with other variables held at near-optimal values.

Results

The ensemble model suggested that at the global scale and resolution of the model suitability for *K. polystachya* was most strongly determined by temperatures of the coldest month and warmest quarter and the climatic moisture index (Table 1, Figure 3). Winter temperatures (Bio6) were optimally around 0-5 °C, while a preference for summer temperatures (Bio10) below 20 °C was apparent. The modelled response to the climatic moisture index indicated a preference for humid conditions in which annual precipitation was at least 70% of potential evapotranspiration.

Global projection of the ensemble model in current climatic conditions indicates that the native and known invaded records all fell within regions predicted to have high suitability (Figure 4). Globally, suitable regions for invasion where the species is not yet present are predicted to occur at high elevations in Africa and South and Central America and in the southern most parts of Australia.

In Europe, the model projects a large region of suitability across western and northern Europe, largely coinciding with places where the species has already established (Figure 5). Additionally, the model indicates potential for further range expansion into regions such as northern Iberia, the British Isles, Scandinavia, the Alps, and the mountains of south east Europe (e.g. Apennines, Dinaric Alps, Carpathians, Caucasus). Uncertainty in this projection is greatest in northeast Europe (Figure 5).

The factors considered by the model to limit suitability vary across Europe in a complex pattern (Figure 6). Broadly speaking, unsuitable parts of southern and eastern Europe were considered to either have too hot summers or to be too dry for the species. In more northerly parts of Europe, the unsuitable regions of France and eastern Germany and Poland were modelled as having too low a climatic moisture index. Since these regions are seemingly thermally suitable, *K. polystacha* might be able to occupy wet micro-habitats such as river banks. Cold winters were only found to be a limiting factor on suitability in northern Scandinavia.

Predictions of the model for the 2070s, under the moderate RCP4.5 and extreme RCP8.5 climate change scenarios, suggest a substantial northwards and uphill retraction of the suitable region, without much gain in suitability in the northernmost regions of Europe (Figure 7-8). This is driven by warmer and drier conditions reducing suitability across northwest Europe.

In terms of Biogeographical Regions (Bundesamt fur Naturschutz (BfN), 2003), the Atlantic and Alpine are predicted most suitable for invasion in the current climate (Figure 9). Under the future climate scenarios, predicted suitability decreases in all regions except the Arctic. Similar patterns are seen for individual EU member states, depending on which Biogeographical Regions they occupy (Figure 10).

Table 1. Summary of the cross-validation predictive performance (AUC) and variable importances of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to ten different background samples of the data.

Algorithm	**AUC**	**In the ensemble**	**Variable importance**				
			Minimum temperature of coldest month	**Mean temperature of warmest quarter**	**Precipitation seasonality**	**Climatic moisture index**	**Human influence index**
GLM	0.9613	yes	45%	33%	2%	17%	3%
GAM	0.9615	yes	44%	34%	2%	17%	3%
ANN	0.9629	yes	47%	23%	1%	22%	7%
GBM	0.9554	no	20%	31%	0%	22%	26%
MARS	0.9630	yes	48%	29%	2%	21%	1%
RF	0.9440	no	25%	29%	8%	18%	20%
Maxent	0.9464	no	32%	26%	13%	20%	9%
Ensemble	**0.9922**		46%	30%	2%	19%	3%

Figure 3. Partial response plots from the fitted models, ordered from most to least important. 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. Variable codes: bio_6 = mean minimum temperature of the coldest month (°C); bio_10 = mean temperature of the warmest quarter (°C); CMI = climatic moisture index; HII= human influence index; bio_15 = precipitation seasonality. Note that CMI and HII are log+1 transformed.

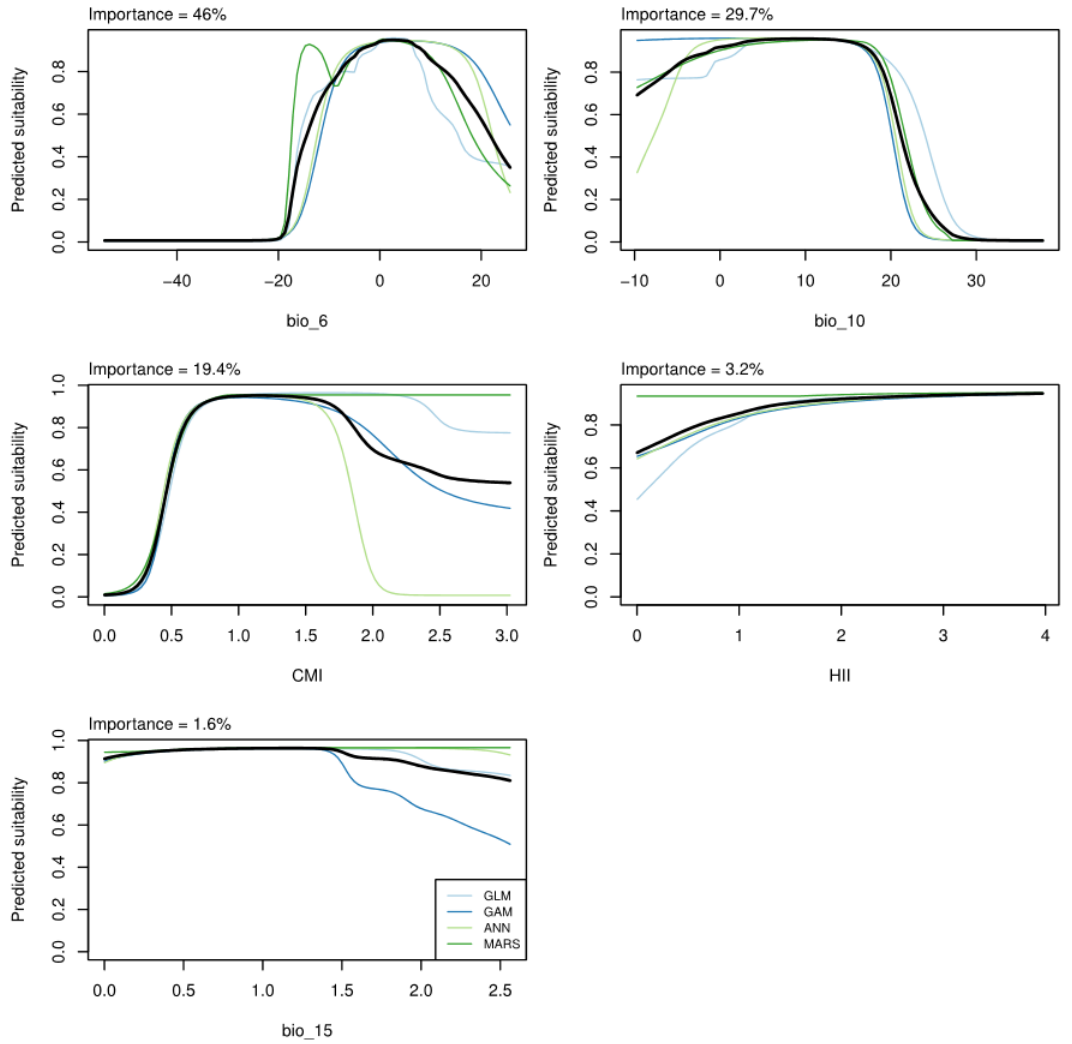

Importance = 46%
Predicted suitability
bio_6
Importance = 29.7%
bio_10
Importance = 19.4%
CMI
Importance = 3.2%
HII
Importance = 1.6%
bio_15
GLM
GAM
ANN
MARS

Figure 4. (a) Projected global suitability for *Koenigia polystachya* 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. Red shading indicates suitability. White areas have climatic conditions outside the range of the training data so were excluded from the projection. (b) Uncertainty in the suitability projections, expressed as the standard deviation of projections from different algorithms in the ensemble model.

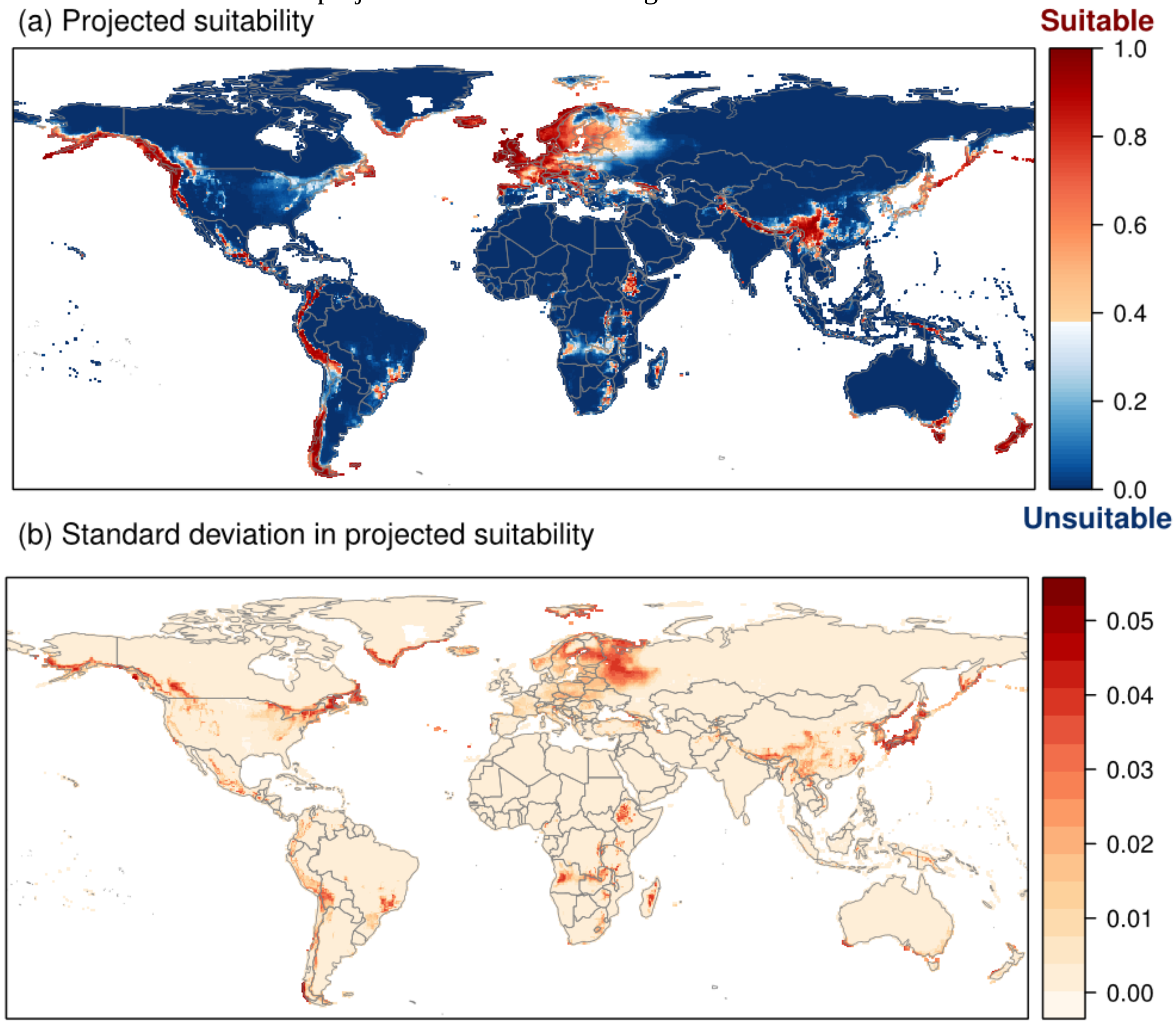


Figure 5. Projected current suitability for *Koenigia polystachya* establishment in Europe and the Mediterranean region. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

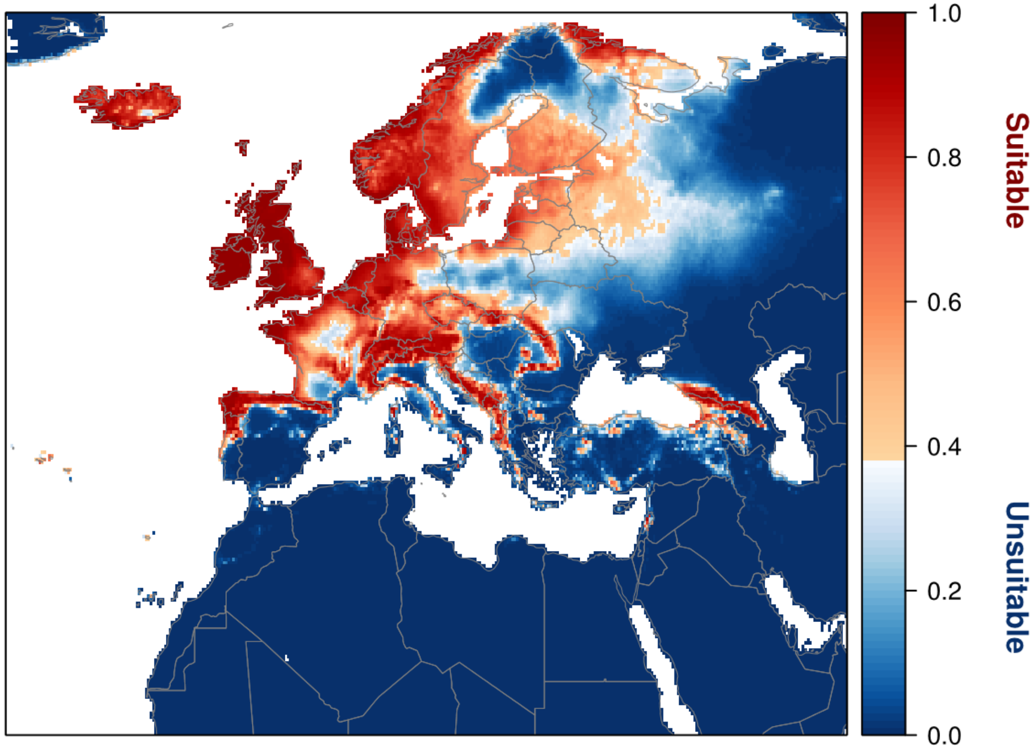


Figure 6. Limiting factor map for *Koenigia polystachya* establishment in Europe and the Mediterranean region in the current climate. Shading shows the predictor variable most strongly limiting projected suitability.

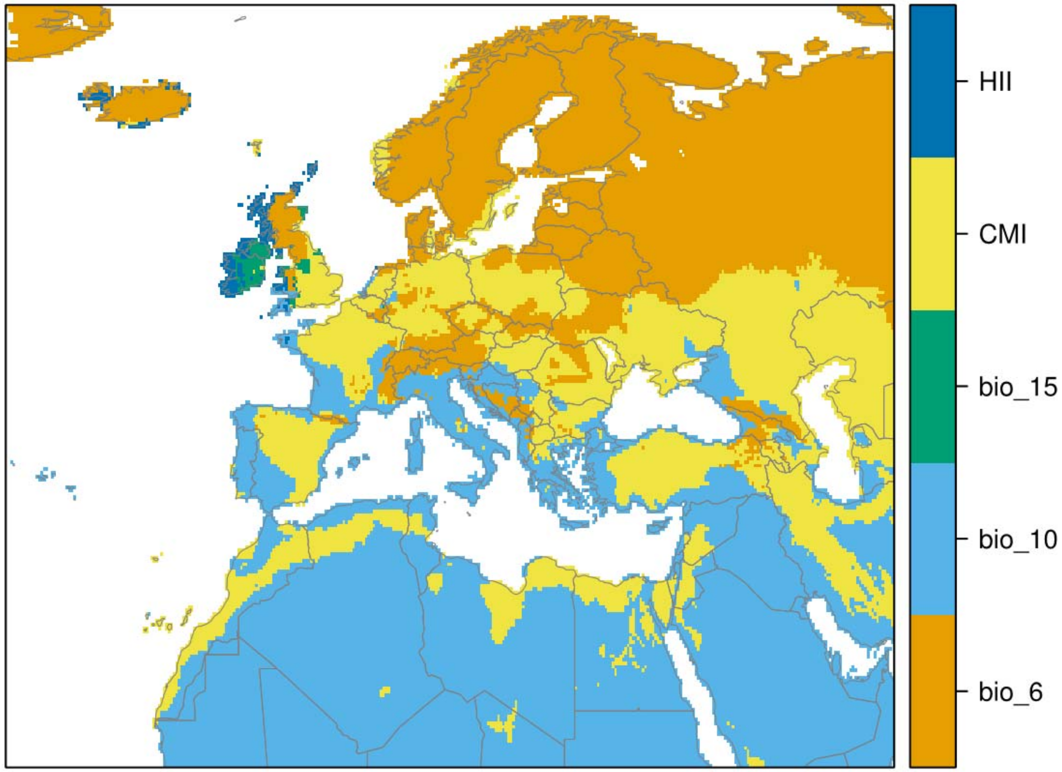


Figure 7. Projected suitability for *Koenigia polystachya* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5.

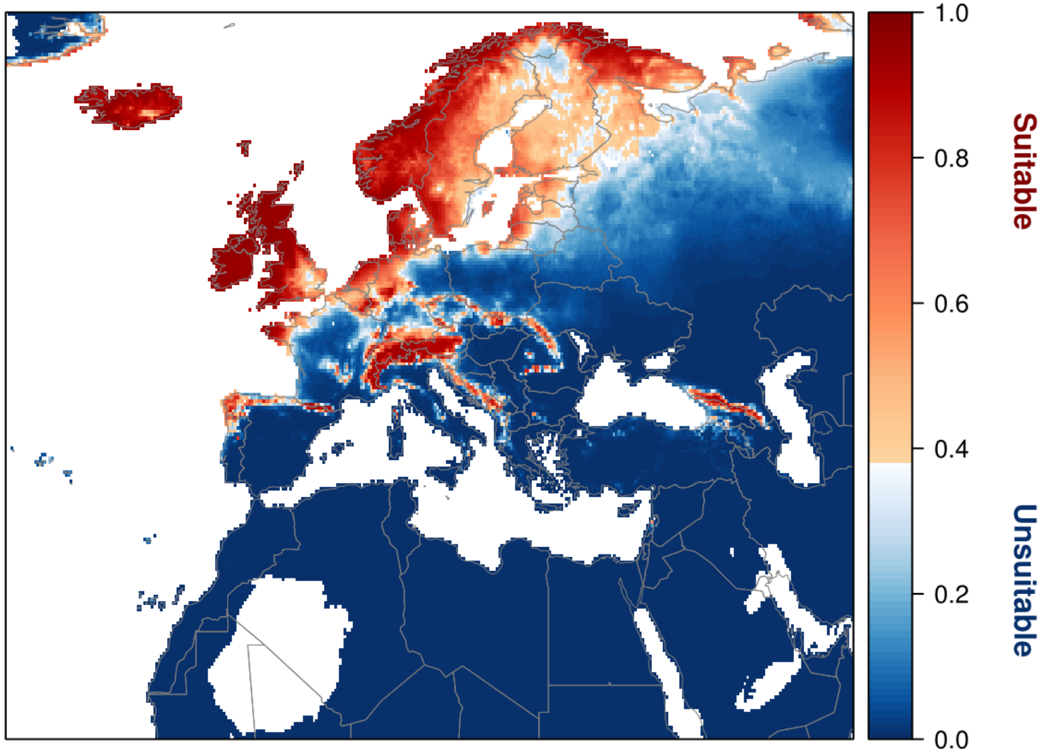


Figure 8. Projected suitability for *Koenigia polystachya* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP8.5, equivalent to Figure 5.

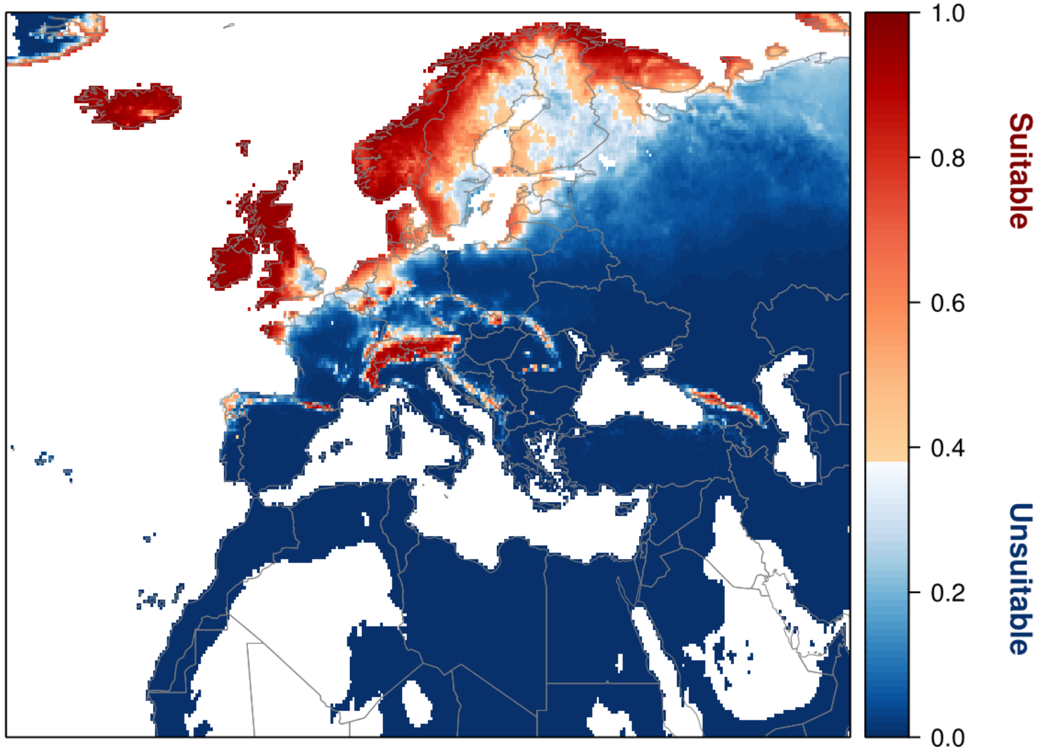
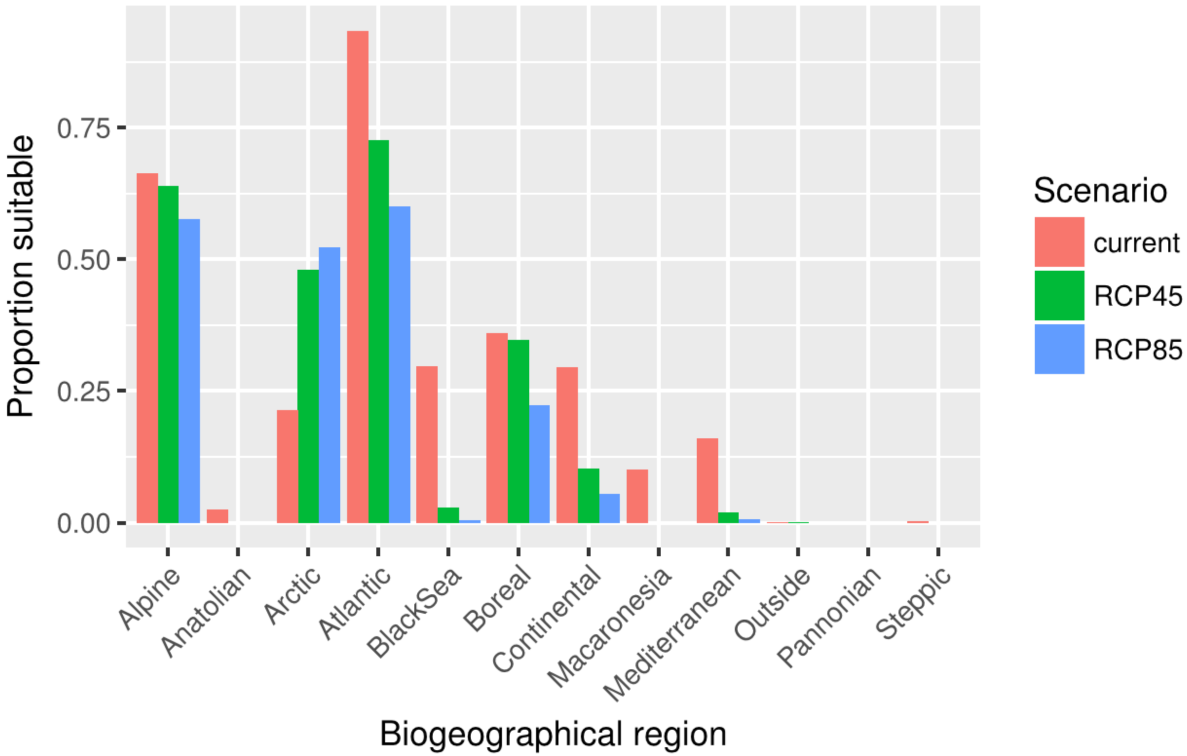

1.0
0.8
0.6
0.4
0.2
0.0
Suitable
Unsuitable

Figure 9. Variation in projected suitability among Biogeographical regions of Europe (Bundesamt fur 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 emissions scenarios RCP4.5 and RCP8.5. The coverage of each region is shown in the map below.



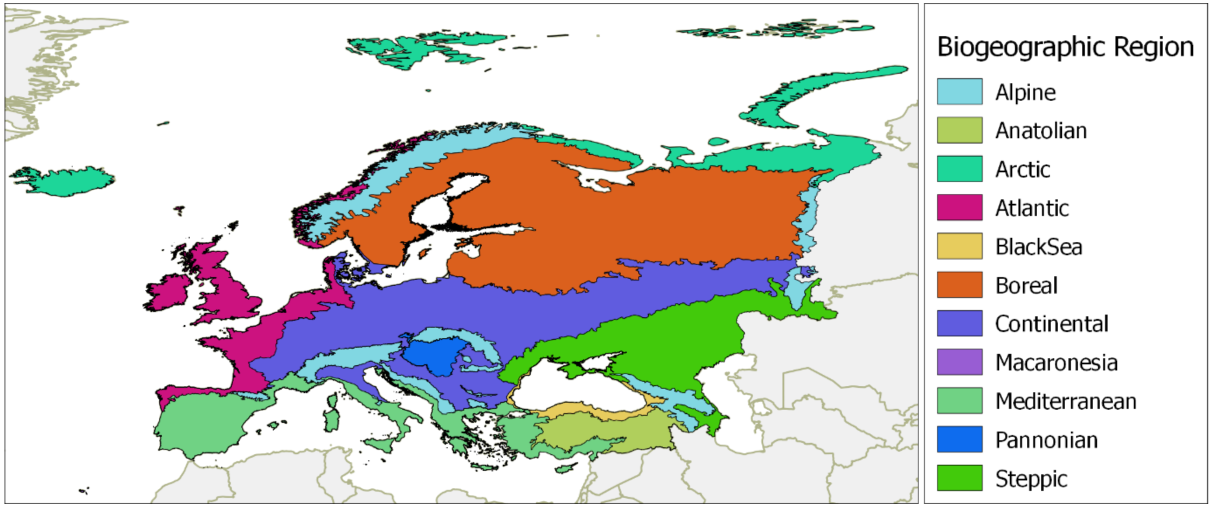
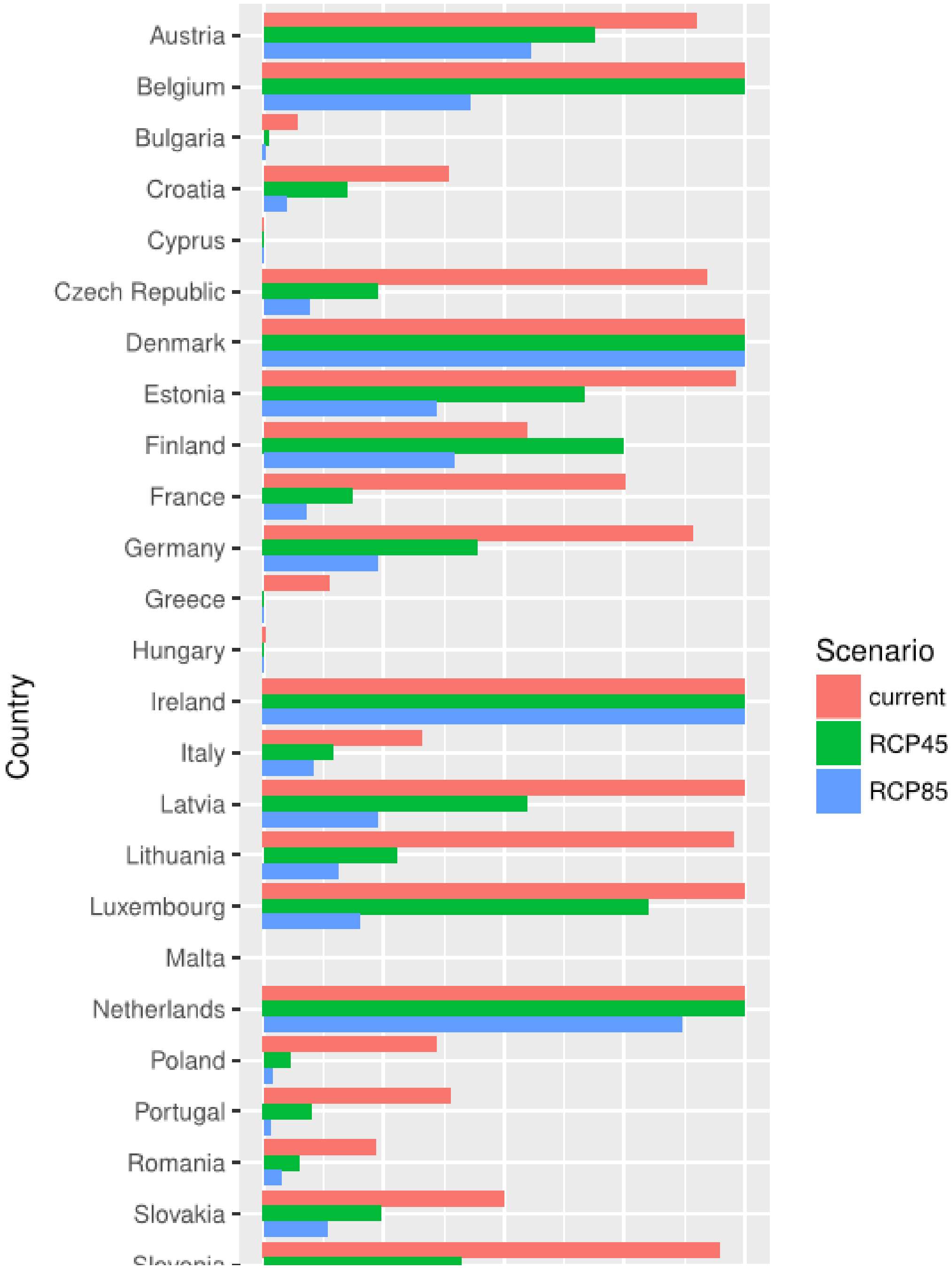

Biogeographic Region
Alpine
Anatolian
Arctic
Atlantic
BlackSea
Boreal
Continental
Macaronesia
Mediterranean
Pannonian
Steppic

Figure 10. Variation in projected suitability among EU28 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 emissions scenarios RCP4.5 and RCP8.5. Malta is excluded as it is outside the predictor grid coverage.


Country
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
Scenario
current
RCP45
RCP85

Caveats to the modelling

Modelling the potential distributions of range-expanding species is always difficult and uncertain.

The modelling here is subject to uncertainty because there was no ecophysiological information available to contribute to definition of the unsuitable background region.

The modelling did not consider other variables potentially affecting occurrence of the species, including soils or biotic interactions.

To reduce the effect of spatial recording biases on the modelling, the selection of the background sample was weighted by the density of vascular plant records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, a number of factors mean this may not be the perfect null model for species recording, especially because additional data sources to GBIF were used.

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Template for Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Koenigia polystachya*** **(Wall. ex Meisn.) T.M.Schust. & Reveal**
Species (common name)	**Himalayan knotweed**
Author(s)	**Rob Tanner**
Date Completed	**19.09.2018**
Reviewer	**Peter Robertson**

Summary

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.

Koenigia polystachya **is already established within the risk assessment area and although the pathways of horticulture (escape from confinement) and transport (contamination (transport of habitat material: soil and vegetation)) 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.**

Although there are no specific studies on the management of ***K. polystachya*** **is can be considered that management practices should follow that of other knotweeds in the family Polygonaceae.** ***K. polystachya*** **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 isolation of patches using thick black plastic etc. Repeated applications are likely to be required to exhaust the rhizome system below-ground. Another option would be the excavation of the rhizome material and dispose of it in a registered land fill.**

Classical biological control has not been evaluated for this species but surveys within the species native range have identified several invertebrate and fungal species which inflict considerable damage on the species (Tanner pers, comm, 2018).

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	One potential pathway for entry or spread of *Koenigia polystachya* 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.	*Koenigia polystachya* is not regarded as a 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. 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 rhizome material as a contaminant of topsoil may also 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 soil between EU Member States, soil management plans, identification guides, factsheets, Codes of	Phytosanitary inspections can be implemented on commodities coming into the EU from outside but the risk of *K. polystachya* entering as a contaminant is moderate. The author could not find any examples where rhizomes have 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

	conduct should be referred too/developed. Preventing the spread of K. polystachya 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 *K. polystachya* should be the priority as eradication can be extremely difficult and expensive (Duncan, 2013).		
Methods to achieve eradication 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	**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. Larger agriculture machinery may be used in more open habitats. CABI (2018) highlight that stems should be cut at least every 2-3 weeks from April until August and this action may need to be repeated for at least two to three years. Mowing alone is unlikely to eliminate the species (DiTomaso et al., 2013). It should be noted that cutting or mowing is not recommended by some (e.g. Emanuel et al., 2011) as this can act to encourage new growth.	High confidence in the assessment

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.			
	Physically covering population	There are no reports that covering alone leading to long-term control of populations and therefore this is not recommended in isolation. DiTomaso et al. (2013) highlights that fabric sheets can have better results in the control of 'knotweed' compared to plastic sheeting.	High confidence in the assessment
	Chemical application (herbicides)	For chemical control CABI (2018) states: to successfully control *K. polystachya* using herbicides, the active ingredient in a herbicide product must have a mode of action designed to move the chemical from the leaves into the root system at sufficient concentrations to kill the root tissue. Herbicides with an active ingredient of glyphosate, triclopyr, 2-4-D, picloram and imazapyr have been shown to be variably effective in controlling knotweeds, either separately or in combinations (Soll, 2004). Chemicals can be applied in varying formats including spraying or stem injections. It resprouts vigorously following cutting, mowing, digging and herbicide treatments, especially early in the growing season, until at least August. Successful eradication of just one patch is likely to take more than one year, and multiple treatments in most cases. Landscape level projects and large sites will almost certainly require integrated herbicide use into the control strategy. There are no costs available for *Koenigia polystachya* control. However, costs for other species within the family Polygonaceae are available and may be similar due to similar form and function.	High confidence in the assessment

		Industry specialists for Japanese knotweed control estimate chemical control can be between 890 – 2 625 EUR (< 49 sq m) to 3 515– 6 185 EUR (100 sqm to 499 sq m) (Japanese knotweed specialists, 2018).	
	Removal of rhizomes and contaminated soil	Excavating the soil with rhizomes can 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. Industry specialists for Japanese knotweed control estimate that this can cost between 3 560 – 8 455 EUR (< 49 sq m) to 9 790 – 17 800 EUR (100 sqm to 499 sq m) when combined with herbicide treatment methods and relocated on site. For full excavation and removal off site (to a landfill), the costs can be: between 3 560 – 17 800 EUR (< 49 sq m) to 31 150 – 106 800 (100 sqm to 499 sq m) (Japanese knotweed specialists, 2018).	Moderate confidence in the assessment
Methods to achieve management		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 is not 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.	High confidence in the assessment
	Covering with thick black plastic or fabric sheets	Covering populations alone is not 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.	High confidence in the assessment
	Chemical control	Chemical control could be considered cost effective when controlling small populations of the species. Repeated applications may be needed to maintain suppression of the population.	High confidence in the assessment
	Excavation of the rhizomes from the soil	Excavation of rhizomes 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 (Japanese knotweed specialists, 2018).	High confidence in the assessment

	Biological control	At present, biological control against *Koenigia polystachya* has not been considered. However, the species has been surveyed within its native range and there are a number of invertebrate and fungal pathogens that exert considerable damage to native populations. 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
	Physical root barriers	Root barriers have been utilized in the containment of Fallopia japonica. This method does not eradicate the species but aims to manage its spread into other areas. This method could be applied to *Koenigia polystachya.* Industry specialists for Japanese knotweed control estimate that this can cost between 1 557 – 4 405 EUR (< 49 sq m) to 8 900 – 31 150 EUR (500 – 1000 sq m) when combined with excavation methods (Japanese knotweed specialists, 2018).	High confidence in the assessment

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S. richetri worker, credits : Alex Wild

Name of organism: *Solenopsis richteri,* Forel (1909).

Author(s) of the assessment:
Olivier Blight, Dr, Institut Méditerranéen de Biodiversité et d'Ecologie, Avignon University, France

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

Peer review 1: Wolfgang Rabitsch, Environment Agency Austria, Vienna, Austria
Peer review 2: Jørgen Eilenberg, University of Copenhagen, Denmark
Peer review 3: Richard Shaw, CABI, UK
Peer review 4: Marc Kenis, CABI, Switzerland

This risk assessment has been peer-reviewed by two independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study.

Date of completion:
10/18/2018

[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

Contents

RISK SUMMARIES

	RESPONSE	CONFIDENCE[i2]	COMMENT
Summarise Entry[3]	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	The most important pathway of introduction for *S. richteri* in Europe is the entry of nests as contaminant of nursery material (including soil) and as stowaway/hitchhiker in container/bulk or other commodities (e.g. vehicles, machinery, packaging material). However, the propagule pressure of nests is largely unknown. Polygyne colonies in South America are mobile and disperse by budding, promoting the chances of queens with workers being transported from this region. Queen ants are also likely to arrive as hitchhikers, but only aircraft will allow the fast transfer that will allow a successful establishment. The entry of *S. richteri* in the EU is scored moderately likely because it has never been intercepted at the Netherlands border (nor has it in Australia, Hawaii or New Zealand and only once in the USA). Moreover, *S. richteri* has a restricted North American distribution. It is more widespread in southern Brazil, Uruguay, and northern Argentina. This assessment of moderately likely risk of entry should be reconsidered in the future if its distribution expands beyond the Americas.
Summarise Establishment[4]	very unlikely unlikely	low **medium** high	Once entered, *S. richteri* is likely to find suitable habitat for nesting in close proximity to sites of arrival. However, there is only limited experimental data on

2 In a scale of low / medium / high, see Annex III

3 In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

4 In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

	moderately likely **likely** very likely		climate tolerances of *S. richteri*. The climate assessment is based principally on consideration of the large body of experimental data relating to *S. invicta*, and on climate estimates from known sites of establishment of *S. richteri*. Species distribution model available for *S. richteri* indicates a total area below 2% of the EU suitable for its establishment. The climate of the Atlantic region is considered suitable for establishment. *S. richteri* is unlikely to encounter natural enemies but would encounter competition from other dominant ants. Its ability to establish at sites dominated by *Linepithema humile* or *Tapinoma magnum* is unknown. It is likely that if established, the ant will have a patchy distribution, with moderate to high densities and extent in open disturbed habitats. This assessment is based on one species distribution model. The use of additional models may improve the prediction and confidence level of this assessment.
Summarise Spread[5]	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	In all potentially infested biogeographical regions, *S. richteri* will probably spread moderately compared to other insects. Suitable habitat occurs in the EU. A range of low vegetation cover habitats are favoured, including urban areas, agricultural land and grasslands; forest is unlikely to be colonised. Colony development is relatively slow, and sub-optimal temperatures are likely to restrict foraging and colony

[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly

			development and extend the period from colony founding to the production of reproductives. Although *S. richteri* can spread by natural means over few kilometres per year, its spread will occur mainly through human-assisted transport, in particular with soil and infested items, but its distribution will be constrained by climate, habitat suitability and competition from other dominant ants.
Summarise Impact[6]	minimal minor **moderate** major massive	**low** medium high	There is currently limited published information on the impacts of *S. richteri* in invaded areas in the USA, which constrains the impact assessment. However, considering its similarity to *S. invicta*, it is likely that the species locally has a moderate to major environmental, economic and social impacts in invaded areas. It has significant medical consequences, even at low ant densities, due to human reactions to the venom. Moreover, the presence of colonies in urban areas can impact negatively outdoor activities and resulting in initiation of pest control. It has some detrimental impacts in agriculture (e.g., stinging domestic stock) and horticulture (e.g., stinging pickers, mounds interfering with equipment) wherever the ant established. Finally, it is likely that *S. richteri* has a negative impact on biodiversity. *Solenopsis richteri* may impact plant/insect interactions by reducing the abundance and richness of local ants and more broadly ground active insects. They may also imperil lizards and birds such as *S. invicta*. The transferability to Europe is hindered by uncertain data on habitat/climatic suitability that may limit the

6 In a scale of minimal / minor / moderate / major / massive, see Annex II

			geographic area that is most favourable to the insect. In other words, if only limited zones in the Atlantic and Continental biogeographical regions will be favourable for the ant, impacts will be largely restricted to these zones.
Conclusion of the risk assessment[7]	low **moderate** high	**low** medium high	*Solenopsis richteri* is not one of the most successful invasive ants on earth but there is no doubt that it can enter Europe through a variety of pathways. However, its limited native and introduced distribution reduces the likelihood of it being accidently transported to Europe. Its establishment and impact will be constrained by climatic, habitat suitability and competition from other dominant ant species. It might become an environmental, economic and social pest in some areas of West Europe, but the extent of its potential distribution remains unclear. This assessment of moderate risk should be reconsidered if its distribution expands beyond the Americas.

[7] In a scale of low / moderate / high

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established (future)*	Invasive (currently)
Austria	-	-	YES	-
Belgium	-	-	-	-
Bulgaria	-	-	-	-
Croatia	-	-		-
Cyprus	-	-	-	-
Czech Republic	-	-	YES	-
Denmark	-	-	-	-
Estonia	-	-	-	-
Finland	-	-	-	-
France	-	-	YES	-
Germany	-	-	YES	-
Greece	-	-		-
Hungary	-	-	-	-
Ireland	-	-	YES	-
Italy	-	-	YES	-
Latvia	-	-	-	-
Lithuania	-	-	-	-
Luxembourg	-	-	-	-
Malta	-	-	-	-
Netherlands	-	-	-	-
Poland	-	-	YES	-
Portugal	-	-	YES	-
Romania	-	-	-	-

Slovakia	-	-	-	-
Slovenia	-	-	YES	-
Spain	-	-	YES	-
Sweden	-	-	-	-
United Kingdom	-	-	YES	-

*Countries with suitability index >0.5 in current climate or foreseeable climate change in Bertelsmeier et al. (2015)

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Alpine	-	-	-	-
Atlantic	-	-	YES	-
Black Sea	-	-	-	-
Boreal	-	-	-	-
Continental	-	-	YES	-
Mediterranean	-	-	YES	-
Pannonian	-	-	-	-
Steppic	-	-	-	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Established (future)	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				

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Scientific name: *Solenopsis richteri* Forel 1909. Class: Insecta Order: Hymenoptera Family: Formicidae Genus: *Solenopsis* Westwood, 1840 *Solenopsis richteri* can be recognized by their large mounds, polymorphic castes (varying sizes of workers), and 10-segmented antennae ending in a 2-segmented club. However, because *S. richteri* hybridizes with *S. invicta*, it can be a challenge to differentiate them from the hybrid, which may have characters of both species. The most reliable methods for identification of this group is a cuticular hydrocarbon test or a genetic analysis. Recently, however, immunoassays have been suggested as a means for discrimination between *S. invicta, S richteri* and hybrids (Valles et al 2018). Original name: *Solenopsis pylades* var. *richteri,* Forel Synonyms: *Solenopsis saevissima* var. *oblongiceps* Santschi, *Solenopsis pylades* var. *tricuspis* Forel, *Solenopsis saevissima* st. *richteri* Forel, *Solenopsis saevissima* var. *tricuspis* Forel. A comprehensive and regularly updated list can be found at www.antweb.org . Common name: Black Imported Fire Ant (BIFA) Due to the limited distribution of *S. richteri* in the USA, there is much more information available on the biology and ecology of *S. invicta*. Where there was a shortage of information on *S. richteri* this is supplemented with information on *S. invicta* in this pest risk assessment, but this is made clear each time.
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	The genus *Solenopsis* contains about 200 species, among which 18 to 20 are “true fire ants”, which all look very similar and have the potential of becoming invasive. Fire ants are a group of related species that has its centre of diversity in southern South America.

wild, in confinement or associated with a pathway of introduction]	Identification of fire ants to species is difficult and usually involves evaluating the morphology of a series of major workers rather than just one specimen. No varieties or breeds of *S. richteri* are known, but hybridization between *Solenopsis* species is regularly observed, particularly between *S. invicta* and *S. richteri*. Hybrid fire ants occupy about 130 000 km^2 in North America, a considerably larger area than remains of *S. richteri* (~30 000 km) in North America (Tschinkel 2006). A regularly updated distribution map can be found at www.antweb.org. The two taxa are still considered separate because they are seen as distantly related within the *S. saevissima* complex by genetic (Ross and Trager 1990) and morphological characterization (Trager 1991). The hybrid taxon is excluded from this assessment. A key for separation of the taxa in the *Solenopsis* species-group was provided by Trager (1991).
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	A risk assessment has been made for fire ants (*Solenopsis* spp.) in the Netherlands, but focused rather on *S. invicta* and *S. geminata* than on *S. richteri*. which concludes that, although they are regularly found during import inspections in the Netherlands, it is unlikely that they could establish outdoors in the country. However, establishment in permanently heated buildings is possible (e.g. *Solenopsis geminata*), and can cause nuisance to humans through their sting and the destruction of equipment such as electrical installations (including air conditioner units, computers, etc.) (Noordijk 2010). *S. richteri* has not been intercepted at the Netherlands border unlike *S. invicta* and *S. geminata*. Another RA relevant for Europe has been carried out for New Zealand, which classified *S. richteri* as having a *low risk* of entry and a *moderate* to *high risk* of establishment and spread (Harris 2005). However, RA made for different regions are not easily comparable.
A4. Where is the organism native?	*Solenopsis richteri* is native to South America, from south-eastern Brazil (Rio Negro, Paraná) west into Misiones province (Trager 1991). The southern part of the range is limited by the Atlantic Ocean on the east and extends west to Mendoza Province and as far south as Uruguay (Lofgren et al. 1975), and Buenos Aires Province in Argentina (Briano and Williams 2002).
A5. What is the global non-native distribution of the organism outside the risk assessment area?	*Solenopsis richteri* has established outside its native range only in the southern USA. Its current distribution, restricted by the presence of *S. invicta* with which it does not co-exist, is an area of about 30,000 km^2 in north-western Alabama, north eastern Mississippi, and southern Tennessee (www.antweb.org). Between S. *richteri* and *S. invicta* is a band of territory occupied by a hybrid between the two species (Trager 1991). *S. richteri* is thought to be more tolerant to cold temperatures and has the capacity to spread to areas marginally suitable for *S. invicta* in the USA (Korzukhin et al. 2001).

A6. In which biogeographical region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established?	Recorded: Not yet recorded or established in the risk assessment area Established: Not yet recorded or established in the risk assessment area
A7. In which biogeographical 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?	<u>Current climate (suitability index above 0.5 in Bertelsmeier et al. (2015), see annexe 1):</u> Atlantic, Continental and Mediterranean biogeographical regions <u>Future climate (suitability index above 0.5 in Bertelsmeier et al. (2015), see annexe 1):</u> Atlantic, Continental, Mediterranean and Alpine biogeographical regions According to the only available species distribution model (Bertelsmeier et al. 2015), *S. richteri* will not establish widely in Europe under both current and future climatic conditions until 2080. However, it will have the capacity to do so in Southern Europe in the Atlantic, the Continental and the Mediterranean biogeographical regions. It is also predicted that the Alpine biogeographical region will be suitable, but with a low habitat suitability index, in 2080. For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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.	Not yet recorded or established in the risk assessment area
A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	According to the only available species distribution model (Bertelsmeier et al. 2015), *S. richteri* is not predicted to establish widely in Europe under both current and future climatic conditions until 2080. The range of habitat suitability is expected to increase in the future (2080) but areas scored with the highest suitability index will decrease. 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. Current climate (suitability index above 0.5 in Bertelsmeier et al. (2015): France, Germany, Ireland, Slovenia, United Kingdom. Future climate (suitability index above 0.5 in Bertelsmeier et al. 2015): Austria, France, Ireland, Italy, Slovenia, United Kingdom.

	Confidence will be increased with other SDM…
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?	Yes. It is considered to be invasive. It has ecological and economic impacts albeit its impacts are restricted to the USA. However some authors do not consider this species as invasive but rather as having the capacity to become invasive (e.g. Peterson and Nakazawa 2008).
A11. In which biogeographical region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	None.
A12. In which EU member states has the species shown signs of invasiveness?	None.
A13. Describe any known socio-economic benefits of the organism.	At present there are no socio-economic benefits in areas where it is invasive. The species is not present in the RA area.

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."
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	none very few few moderate number **many** very many	low medium **high**	Ants can be dispersed through many different pathways (Suarez et al. 2005).

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

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

1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	a) Transport-Stowaway (Hitchhikers in or on airplane) 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.)		*Solenopsis richteri* is termed a "tramp" ant, it can hitchhike with many commodities through many pathways. However, only the entry of queen ants and nests present a risk of establishment. In the case of an independent colony foundation, the queen has to find a suitable place quickly after the nuptial flight. These restrictions reduce the number of active pathways as the risk of predation is very high. *S. richteri* has only invaded the USA, so data of potential pathways of introduction are lacking. It has not been intercepted in New Zealand, nor in Australia, Hawaii or the Netherlands. Harris (2005) provides a very detailed analysis of potential pathways of introduction of *S. richteri* in New Zealand, which is also relevant for Europe.
Pathway name:	a) Transport-Stowaway (Hitchhikers in or on airplane)		
1.3a. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional **unintentional**	low medium **high**	This concerns only newly-mated queens that are transported few hours after mating.
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year?	very unlikely **unlikely** moderately likely likely very likely	**low** medium high	Although many individuals may travel this pathway, new colonies are established by solitary fertile queens following a mating flight. Queens seek moist areas within a few kilometres of the parent colony. Once a

Subnote: In your comment 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.			suitable site is found the female sheds her wings and digs a small burrow into the soil and seals it. Although few data is available on ant interceptions at ports and airports, the proportion of queens in interception database is very low which suggests a relatively low number of newly-mated queens travelling along this pathway. Limited data is available in US, New Zealand and Australia. The proportion of queens in these data base is very low and not routinely recorded by plan healthy inspectors in EU ports of entry. So these database are not informative.
1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely **unlikely** moderately likely likely very likely	**low** medium high	Queen ants are able to survive several tens of days using their own reserves before the first workers emerge when they are hidden in their nest with humidity. However, their chance of survival and of establishing a nest decreases in nature after few hours if they are not settled in a nest. Considering that ships from the nearest infested areas take more than a week to reach the EU, newly-mated queens might only arrive successfully in airplanes. However, it cannot be ruled out that newly-mated queens establish a nest on a ship if they find suitable conditions (see Qu. 1.5a). Multiplication and the establishment of a small nest during an intercontinental flight however is highly unlikely.
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	N/A. There are no management practices against hitchhiking ants or ant queens in or on airplanes in place.
1.7a. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely**	low medium **high**	Solitary queens or even several queens or small nests are not easy to detect in cargo planes and thus their detection rates will be low.

	very likely		
1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Nuptial flights of *S. richteri* occur at air temperature as low as 21°C (Lofgren et al. 1975). In *S. invicta* flights can occur all year in subtropical areas but predominantly occur in late summer (May through August in North America/USA) when climate conditions are most suitable and soil temperatures optimal (Lofgren et al. 1975). In Europe a relatively narrow window of suitable conditions is likely for nuptial flights. However, commodities with which ants can enter Europe are imported throughout the year.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Many airports are surrounded by suitable habitats including irrigated/watered gardens and parks. Indeed, this species simply requires soil as a substrate in which to establish a nest and has been found to occur in diverse open areas of pastures, cultivated fields, and lawns (Taber 2000).
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	The likelihood is scored moderately likely because the number of queen ants travelling through this pathway is expected to be relatively low and the duration of the transportation would be unlikely to favour the survival of the queen. Harris (2005) scored the likelihood of introduction of a *S. richteri* queen ant by aircraft as "low".
Pathway name:	*b)* Transport-Contaminant (nursery material and other matters from the horticultural trade)		
1.3b. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	This concerns both fully developed nests (with active workers) and newly-founded nests (before workers are developed and start foraging) transported in nursery material by the horticultural trade. Newly-founded nests can also be formed by queens transported in ships before the nursery material arrives at destination.

1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	There are no data on *S. richteri* nests arriving through the horticultural trade in Europe, nor in the USA, in New Zealand and in Australia. 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 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. Flower pots are one of the preferred habitats for *S. invicta* 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 harbour ant nests. Both polygynous and monogynous nests occur in *S. richteri*. Polygynous colonies are particularly large since they include many queens and may contain thousands of workers. The maximum size of a fully developed colony of *S. invicta* may reach more than 200,000 workers (Tschinkel 2006). In *S. richteri* (and other members of the *S. saevissima* species group) specific amino acid substitutions in a gene are associated with the expression of monogyny or polygyny (Ross et al. 2003). Approximately half the *S. richteri* colonies examined in San Eladio, Argentina were polygyne with up to 180 queens (Calcaterra et al. 1999). Only monogyne colonies have been found in the USA (Vogt et al. 2004), which suggests that either only the monogyne form has been introduced, or, if the polygyne form did establish, it has subsequently disappeared.

			Ant nests might get into this pathway in large numbers as contaminants of horticultural materials including soil. The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place. NB: The number of ports of origin are limited.
1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Ant queens are able to survive several tens of days using their own reserves before the first workers emerge. Once sealed in a newly-founded nest, a *S. invicta* queen is able to survive 13 to 95 days, i.e. much longer than before nest establishment (Markin et al. 1972). Likelihood of survival nevertheless will decrease with increasing travel duration. Multiplication of a small nest during intercontinental translocation however is highly unlikely.
1.6b How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely **likely** very likely	**low** medium high	Horticulture plants and soils/substrates are usually chemically treated before shipment but can be infested after treatment either before departure or during transport.
1.7b. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	Fully developed nests are quite visible. Newly-founded nests with few queen(s) and workers in the soil/substrate can easily arrive undetected.
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	The horticultural trade is active throughout the year.

1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Potted plants and plant materials are likely to be transported outdoors in gardens, which may be, or adjoin, a suitable habitat. It is expected that suburban and urban habitats are most at risk at the beginning of an invasion
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely **moderately likely** likely very likely	low medium **high**	We consider this pathway as the most likely pathway of entry of *S. richteri* into Europe. Noordijk (2010) also consider the horticultural trade as the most likely pathway for introduction of *Solenopsis* species in the Netherlands.
Pathway name:	c) Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)		
1.3c. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	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 and fully developed nests. 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 electric equipment.
1.4c. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	There are no data on *S. richteri* nests arriving in Europe. Sea containers and all articles of commerce cited above were scored by Harris (2005) as presenting a high likelihood of introduction for nests of *Solenopsis* species. Ants are not listed as quarantine pests in the EU and, therefore, records rarely appear in the national and international lists of intercepted pests. Polygynous nests include many queens and may contain thousands of workers. The maximum size of a fully developed colony of *S. invicta* may reach more than 200,000 workers (Tschinkel 2006). Ant nests might get

			onto the pathway in large numbers as stowaway in containers or other bulk freight, including soil. The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.
1.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Ant queens are able to survive several tens of days using their own reserves before the first workers emerge. Once sealed in a newly-founded nest, a *S. invicta* queen is able to survive 13 to 95 days on her own reserves, i.e. much longer than before nest establishment (Markin et al. 1972). This is sufficient to survive longer trips to Europe from any origin. Likelihood of survival nevertheless will decrease with increasing travel duration.
1.6c How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	In most of the commodities in this pathway, there are no management practices in place.
1.7c. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Many of these commodities are not carefully inspected. While established nests are usually obvious, newly-founded nests are often inconspicuous. Newly-founded nests with few queen(s) and workers could easily arrive undetected.
1.8c. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Commodities that can carry *S. richteri* are introduced to the risk assessment area throughout the year.
1.9c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Several of the potential commodities and items in which nests can hide can be transported to suitable habitats since the ant particularly likes open and disturbed habitats, which are found everywhere, specifically in urban and semi-urban areas.

1.10c. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Given the high numbers and types of containers, commodities and items that can be associated with *S. richteri*, entry along pathway can be considered as being moderately likely.
End of pathway assessment, repeat as necessary			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	The species has never been recorded/intercepted in Europe. Its distribution in the native range (South America) and the introduced range (a limited part of the USA) decreases the likelihood of it being accidently introduced into Europe. It is moderately likely that this will happen in the future, specifically with contaminated soil in the horticultural trade and/or as stowaway with container/bulk imports in sea or air freights. This scoring should be reconsidered in the case of an expansion of the introduced range of *S. richteri*.
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Climate change is not changing the risk of introduction or likelihood of entry based on the mentioned active pathways except, for example, if shipments of horticultural plants from invaded areas increase.

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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely **likely** very likely	Low **medium** high	Only one climatic model has been developed for *S. richteri* at a global scale (Bertelsmeier et al. 2015). Using a climate matching model (Maxent) based on present distributions, they showed that less than 2% of the European continent is presently suitable for *S. richteri*, but predicted a potential distribution mainly in France, Ireland, United Kingdom, and Germany (Supplementary material, Fig. A2). A climatic model developed specifically for France confirms the suitability of these biogeographical regions (Atlantic and Continental) for *S. richteri* (Bertelsmeier and Courchamp 2014). However, Europe is less suitable than the introduced range of *S. richteri* in the USA (Bertelsmeier et al. 2015). Although *S. richteri* seems to be more tolerant to cold temperatures than *S. invicta*, various climatic models developed for *S. invicta* can be used to assess the likelihood of establishment of *S. richteri* in Europe. However, they do not all agree in their conclusions. Morrison et al. (2004) used the model of Korzukhin et al. (2001) to map suitable areas for the reproduction of *S. invicta* worldwide. The model

			used a dynamic, ecophysiological model of colony growth, superposing temperature and precipitation requirements to predict the potential global range distribution of the ant. The model showed that large parts of the Mediterranean region fall in the area suitable for *S. invicta* establishment. Sutherst and Maywald (2005) used the CLIMEX climate modeling software to assess the potential geographic range of *S. invicta* based on an ecoclimatic index (EI). For Europe, the analysis showed that climate per se will not constrain the ant from colonizing countries bordering the Mediterranean and western France. Irrigation would allow it to establish in arid zones and increase colony growth in Mediterranean climates (Supplementary material, Fig. S2, but see Fig S3). However, EI for Europe was significantly lower than for the regions where the ant is highly invasive (e.g. in North America and East Asia), suggesting that, in Europe, establishment and population growth may be less straightforward, except in irrigated lands and in habitats in direct contact with permanent water bodies. Indeed, the model shows much higher EIs when irrigation is added.
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Other abiotic conditions should not be a constraint for the establishment of *S. richteri* in Europe, maybe except for high-altitude environments. The ant prefers disturbed soils, which are found everywhere, specifically in urban and semi-urban habitats.

1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	very isolated isolated moderately widespread **widespread** ubiquitous	low medium **high**	*Solenopsis richteri* prefers open and disturbed habitats, which are found everywhere in Europe. In Argentina, near the southern extent of its natural range, foraging did not occur during the colder months, and in summer workers were seen when air temperature ranged from 19 to 36°C (Palomo et al. 2003). In regions with unsuitable climates, it may survive under artificial warm conditions indoors, in buildings or greenhouses as well as in gardens and parks in cities. *Solenopsis* species have shown temporary indoor colony establishments including at least once in the Netherlands (*i.e. S. geminata*) (Noordijk 2010). However, indoor colonies can normally be eradicated easily.
1.16. 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?	**NA** very unlikely unlikely moderately likely likely very likely	low medium **high**	*Solenopsis richteri* does not require another species for establishment.
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Within its native range *S. richteri* can attain high densities and be dominant in disturbed habitats. *Solenopsis richteri* monopolized space and food in grassland habitat in Argentina that was susceptible to flooding (Folgarait et al. 2004). It can reach high mound densities (707 nests/ha) which is comparable to those seen for *S. invicta* in the USA (Folgarait et al. 2004). There is probably intense competition with other dominant species in some locations. However, *S. richteri* seems to be less competitive than *S. invicta* which has drastically reduced its distribution in the USA (Tschinkel 2006).

			In several suitable areas in Europe, it will have to face the competition at least with two invasive species already established, 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 colonies of many hundred thousands of individuals. Successful colony founding by *S. richteri* within established populations of either species would seem unlikely. The Argentine ant was superior to the highly competitive *S. invicta* during asymmetrical confrontation tests (numerical advantage for the Argentine ant) under laboratory confrontations (Kabashima et al 2007). The Argentine ant is largely distributed along the Mediterranean coast from Portugal to Italy through Spain and France. It has been also recorded in Malta and Greece. Nonetheless, where these competitive species are not present the establishment may easily occur.
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	Only few *Solenopsis* spp. are native to Europe, and no specific natural enemies of *Solenopsis* spp. 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.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	No specific management practices are in place against invasive ants in the wild in Europe. Eradication of single nests is straightforward in buildings (e.g. Noordijk 2010) but much less so outdoors. However, some eradication programmes of *S. invicta* have succeeded, e.g. in Australia (Hoffmann et al. 2016; Wylie et al. 2016)

1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	There are no specific management practices against invasive ants in the risk assessment area. But based on what is done locally to control ants, i.e. chemical treatments, it is unlikely that management practices, if set up, facilitate establishment.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	The eradication of invasive ants outdoors is difficult, especially when populations reach high densities of nests and individuals (Hoffmann et al. 2016). However incipient ant colonies can be successfully eradicated (Hoffmann et al. 2016).
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	*Solenopsis richteri* has monogynous and polygynous populations. The polygynous form can more easily establish because the higher number of queens increases reproduction potential, especially in the critical early stages of establishment. The number of workers in a polygynous nest can vary enormously, from thousands to hundreds of thousands (Taber 2000). However only monogynous colonies have been observed so far in the introduced range of *S. richteri*. It is unknown if establishment of the polygyne form outside its native range would see similar increases in densities and impacts as *S. invicta* in the USA, but they can achieve high densities in ideal conditions within their native range (Calcaterra et al. 1999). Few data are available on the biology of *S. richteri*. Inseminated females (queens) of *Solenopsis invicta* lay up to 200 eggs per hour (Tschinkel 1988). Within one year, the colony can grow to several thousands of workers, within three years it can reach up to 230,000 workers (Tschinkel 1988).

			The peculiar, almost unique, reproductive caste system of these eusocial insects can facilitate establishment. For the Argentine ant, 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).
1.23. How likely is the adaptability of the organism to facilitate its establishment?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	*Solenopsis richteri* is one of the less successful invasive ants (established and invasive only in the USA) which might, among other explanations, suggest a moderate adaptability to new environments. Despite *S. richteri* being a generalist, opportunistic species, it requires open places, especially those that are related to humans. Also, it has a restricted flight period. Nuptial flights have been recorded only during the warmest seasons of the year. However, *S. invicta* which is closely related to *S. richteri* has demonstrated a high adaptability to new environments (Tschinkel 2006).
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Most invasive ants, which are among the most invasive insects worldwide, establish following the entry of single nests or queens (Holway et al. 2002; Vonshak et al. 2010). Therefore, low genetic diversity does not seem to be a barrier to establishment.
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	*Solenopsis richteri* has been introduced (according to interception records) and become established only in Southern US. However, it shares several biological and ecological features with closely-related species such as *S.*

			geminata and *S. invicta* that are two of the most widely distributed invasive ants. Should the climate of Southern Europe be suitable and habitats available for the species, the history of invasion suggests that it is moderately likely to establish in Europe.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	very unlikely unlikely **moderately likely** likely very likely	low medium **high**	As shown with interception data from countries such as the Netherlands (Noordijk 2010), US (Suarez et al. 2005; Bertelsmeier et al. 2018), New Zealand (Harris 2005), *Solenopsis* spp. are regularly intercepted at ports and airports. However, in most cases, these are sterile workers that cannot establish in the wild. Ants are not listed as quarantine pests in the EU and, therefore, interception data are not good indicators of their frequency of entry because they do not have to be mentioned in the national and international lists of intercepted pests. It has to be assumed that there is a considerable number of unreported cases even for *S. richteri* which is absent from almost all interceptions data bases.
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	In the Atlantic and continental biogeographical regions, establishment under current conditions is likely, at least in France, Germany, Ireland, Slovenia, United Kingdom (Bertelsmeier et al. 2015). The absence of other, more regional, models predicting *S. richteri*'s possible distribution in Europe limits our conclusions.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	very unlikely unlikely moderately likely **likely**	low **medium** high	Bertelsmeier et al. (2015) predict an expansion of the potential range of *S. richteri* but the proportion of regions scored with a high suitability index decreases. Under foreseeable climate change, *S.*

	very likely		*richteri* may establish in the Atlantic, Mediterranean, Continental and Alpine biogeographical regions. 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. The absence of other, more regional, models predicting *S. richteri*'s possible distribution in Europe limits our conclusions.

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	minimal minor **moderate** major massive	low medium **high**	New colonies are founded by winged females, capable of flying long distances. This allows new sites of infestation to be established a long distance from the source infestation (Holway et al. 2002).Nuptial flights will result in rapid spread outwards from a site of establishment. Most queens of *S. invicta* do not fly far from the colony of origin but some may fly up to 12 kilometres (Tschinkel 2006). Nuptial flights occur during the warmest seasons of the year. Polygynous colonies can also spread by "budding", i.e. alates mate in the nest and queens disperse only short distances and take workers with her to start a new colony (Tschinkel 2006). Such a strategy does not allow a rapid spread but increase survival rates of queens and colonies. When *S. invicta* colonies reach about 10% of their maximum size they begin producing reproductives (Tschinkel 1988). Under ideal conditions, this can occur within 6 months of founding (Vinson & Greenberg 1986). At suboptimal temperatures this may take longer to achieve, as development rates are strongly temperature dependent (Porter 1988). Colonies budded from polygyne colonies will likely

			produce reproductives sooner than independently founded nests (Tschinkel 1988). Sometimes, an entire colony of *S. invicta* can disperse by rafting/floating on water, e.g. after flooding of its habitat (e.g. Adams et al. 2011). The question is scored "moderate" because it is likely to spread more slowly by natural means than by human assistance.
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	minimal minor moderate **major** massive	low medium **high**	Human assisted pathways of spread are the agricultural and horticultural trade of plants, plant materials, and soil/substrate as well as other movements of commodities. a Invasive ants are commonly transported with horticultural plants (commercial or private). This pathway is probably the main mechanism for human-assisted spread of invasive ants. Building construction or agricultural activities can also contribute to their spread, especially when soil is excavated and moved to different places. Finally, ants can be accidently transported by individuals. For example, invasive ants are known to enter into vehicles, probably because of the heat produce by the engine.
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	a) Transport-Contaminant (Contaminant nursery material) b) Transport-Stowaway (Container/bulk, including road		

	transport, sea freight, airfreight, train, etc.) c) Unaided (Natural dispersal)		
Pathway name:	a) Transport-Contaminant (Contaminant nursery material)		
2.3a. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	
2.4a. 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?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Within Europe, movements of potted plants are unrestricted and frequent. Soil/substrate in potted plants is a favourite media for nesting (see entry section 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 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 contaminant of horticultural materials including soil. The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.
2.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Once sealed in a newly-founded nest, a queen of *S. invicta* is able to survive 13 to 95 days on her own reserves, i.e. much longer than before nest establishment (Markin et al. 1972; Porter 1988). Likelihood of survival is high, nevertheless will decrease with increasing travel duration. Multiplication of a colony during spread within the EU cannot be ruled out, but is rather unlikely.

2.6a. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Horticultural plants and products and soils/substrates are usually not treated before translocation within the EU.
2.7a. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Fully developed nests are quite visible. In contrast, newly-founded nests with few queen(s) and workers can easily travel undetected in soil or other horticultural products.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	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 spread facilitates occurrences in urban, suburban and agricultural habitats.
2.9a. Estimate the overall potential for rate of spread within the Union based on this pathway (when possible provide quantitative data)?	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	We consider this pathway as the most likely pathway of spread of *S. richteri* within Europe. A similar conclusion has been made for New Zealand (Harris 2005). The rate of spread will depend on the internal volume of trade within Europe.
Pathway name:	b) Transport-Stowaway (Container/bulk, including road transport, sea freight, airfreight, train, etc.)		
2.3b. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	Virtually any article of commerce can host hitchhiking ants with 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.

2.4b. 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?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	There are very limited data on ant nests translocated within the EU. Ant nests might be established in transported items in large numbers as stowaways. The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.
2.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Once sealed in a newly-founded nest, a queen of *S. invicta* is able to survive 13 to 95 days on her own reserves, i.e. much longer than before nest establishment (Markin et al. 1972; Porter 1988). Likelihood of survival is high, nevertheless 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.
2.6b. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Most potential commodities that can carry ants or nests are not managed.
2.7b. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	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.
2.8b. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Several of the potential commodities and items in which nests can hide can be transported to suitable outdoor habitats since the ant prefers disturbed soils, which are found everywhere and are often close to storage facilities where commodities may be shipped ,

			specifically in urban, semi-urban and agricultural habitats.
2.9b. Estimate the overall potential for rate of spread within the Union based on this pathway (when possible provide quantitative data)?	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	Given the high numbers and types of commodities and items that can be associated with *S. richteri,* 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. 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).
Pathway name:	c) Unaided (Natural dispersal)		
2.3c. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	
2.4c. 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?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Spread by nuptial flights occur only during the warmest months of the year, and likely will be restricted to few weeks in the risk assessment area; it will include small numbers of alates, while budding usually includes a larger number of queens and workers. After mating, queens fly 3-5 m above the ground. It is possible that reproductives from monogyne colonies form mating swarms fly much higher, as is reported for *S. invicta* (Markin et al. 1971), and therefore could experience enhanced wind-assisted dispersal. The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.

2.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Rates of survival of mated queens are relatively low after the nuptial flight (Hölldobler and Wilson 1990). However, this is compensated by the production of hundreds of females per nest giving a very likely score. Dispersion by budding increases queen survival but reduces dispersion distances.
2.6c. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Management practices during unaided spread are not currently in place.
2.7c. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely **moderately likely** likely very likely	low medium **high**	Low ant densities (e.g. single queens, 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 *S. richteri* has a painful sting and is highly likely to be found in close association with urban areas should aid early detection of its presence, even if its initial establishment may go unnoticed.
2.8c. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Queens of *S. invicta* can fly up to 16 km in extreme cases and will very likely find suitable habitats (e.g. open and disturbed habitat).
2.9c. Estimate the overall potential for rate of spread within the Union based on this pathway (when possible provide quantitative data)?	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	*Solenopsis richteri* should be able to spread unaided to all suitable habitats within its suitable climatic range. Alate females of *S. invicta* can fly up to 16 km and colonies can also be occasionally transported by water flood. This rate of spread decreases in polygynous colonies that reproduce by budding (below 300m per year, Hölldobler& Wilson 1990). For polygyne *S. invicta*,

			the invasion front moved 10.40 m/yr in central Texas via budding (Porter 1988). There are a number of intrinsic and extrinsic factors that influence spread including availability of disturbed habitats and morphology of the queens (Tschinkel 2006; King and Tschinkel 2008).
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	very easy easy with some difficulty difficult **very difficult**	low medium **high**	It will probably be very difficult to physically contain the species. Its spread will be constrained by climate, habitat suitability and competition from other dominant ants. If *S. richteri* becomes established in a European region, quarantine measures could be put in place to restrict the risk of long-distance spread, e.g. through nursery stock, as in USA for *S. invicta* (USDA 2015).
2.11. Estimate the overall potential for rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	Based on observations in North America and the lower ecoclimatic suitability in Europe, we can estimate that it will spread to all potentially infested biogeographical regions, but possibly slower than in North America. Habitat suitability is predicted to be lower in Europe even in relevant biogeographical regions, than in the introduced range of *S. richteri*. 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 depend on the internal volume of trade within Europe.
2.12. Estimate the overall potential for rate of spread in relevant biogeographical regions in foreseeable climate change conditions	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	Climate change will not increase the potential or rapidity of spread directly, but may facilitate population growth with subsequently increasing potential for spread. Despite climate change may widen the distribution range of this species, future

			suitable areas are predicted to have low suitability index (Bertelsmeier et al. 2015).

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is 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?	minimal minor **moderate** major massive	**low** medium high	There is no research on impacts of *S. richteri*, principally due to its limited distribution and displacement by *S. invicta* in the USA. Wang et al. (2013) provided an extensive review of studies on the environmental impact of *S. invicta* since its invasion in China. -Impact on fauna: In southern North America, *S. invicta* threatens several arthropods, molluscs, reptiles, birds, amphibians and mammals by direct predation, competition or stinging (see review by Wojcik et al. (2001), Holway et al. (2002), Allen et al. (2004) and more recent studies such as Allen et al. (2017)). In particular, it has been shown to displace or reduce populations of native and invasive ants (including the Argentine ant) (McGlynn 1999; Holway et al. 2002; King and Tschinkel 2008). It also attacks beneficial

			insects such as parasitoids and predators (Eubanks et al. 2002; Ness 2003). It must be noted, however, that data on direct effects on long term population declines of animals are largely lacking, even for impact on native ants. *Solenopsis invicta* mainly occupies niches in highly disturbed habitats and, in such situations, it is difficult to distinguish between the effects of disturbance and the effects of *S. invicta* on other ants (King and Tschinkel 2006). -<u>Impact on plants</u>: the impact on wild plants has been less studied than that on animals or cultivated plants. However, the flora can also be affected through various mechanisms, such as changes in soil properties (Lafleur et al. 2005), predation or tending of plant pests, direct seed predation and competition with native ant dispersers (Ness and Bronstein 2004). However, *S. invicta* may also facilitate seed dispersal (Stuble et al. 2010). -<u>Alteration of ecosystem functions</u>: Nest building and foraging activities of *S. invicta*, affect physical and chemical soil properties and strongly enhances plant growth through the increase of $NH4^{+}$ (Lafleur et al. 2005). It also affects mutualistic interactions between plants and insects by reducing numbers of plant mutualists that protect the plant or disperse plant seeds (Ness and Bronstein 2004). It is likely that impact on ecosystem functions may be locally major and similar to that observed in presently invaded areas elsewhere.
2.14. 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	minimal minor moderate major	low medium high	N/A. Because the species is not present in Europe, there is no current impact on biodiversity and related ecosystem services.

communities, hybridisation) in the risk assessment area (include any past impact in your response)?	massive		
2.15. 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?	minimal minor **moderate** major massive	**low** medium high	It is likely that, if *S. richteri* establish and spread in the Atlantic and Continental biogeographical regions, the impact on native biodiversity, in particular on arthropods, and small vertebrates would be major and similar to the impacts of *S. invicta*. The magnitude of the impacts will depend on the densities *S. richteri* achieves. Establishment in areas of suboptimal climate will limit dense populations and reduce impacts.
2.16. 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?	minimal minor moderate major massive	low medium high	N/A. Because the species is not present in Europe, there is no current impact on the conservation value of native species or habitats
2.17. 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?	minimal **minor** moderate major massive	**low** medium high	Although *S. richteri* can inhabit a wide range of habitats, in the USA it particularly dominates highly disturbed habitats, such as roadsides, agricultural areas including irrigated soils, gardens, etc. Therefore, many natural habitats of high conservation value may not be threatened by the ant.
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	minimal minor **moderate** major massive	**low** medium high	There is no research on impacts of *S. richteri*, principally due to its limited distribution and displacement by *S. invicta* in the USA. <u>Provisioning-Nutrition</u>: *S. invicta* damages cultivated field crops by feeding on the seeds, seedlings and developing fruit (Adams et al. 1983). It also negatively affects cattle farming (Teal et al. 1999). <u>Regulating-Seed dispersal</u>: *S. invita* may interfere with seed dispersal of native ant species and directly predate (and therefore reduce) amount of seeds (Ness and

			Bronstein 2004). However, *S. invicta* may also facilitate seed dispersal (Stuble et al. 2010). Regulating-Pest and disease Control: *S. invicta* may interfere with beneficial insects that exert biocontrol activities in modified habitats. Cultural-Physical use of landscapes: *S. invicta* is a social nuisance in infested areas. Public areas such as parks and recreational areas may become unsafe for children and people have modified their behaviour to avoid the nuisance (Wylie and Janssen-May 2017).
2.19. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographical regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?	minimal minor moderate major massive	low medium high	N/A. Because the species is not present in Europe, there is no current impact on ecosystem services.
2.20. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographical regions or marine sub-regions where the species can establish in the risk assessment area in the future?	minimal minor **moderate** major massive	**low** medium high	It is likely that, if *S. richteri* finds suitable habitats and climates for its development in the Atlantic and continental regions, the impact on ecosystem services may be locally major and similar to the impacts of *S. invicta*. But its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability. The magnitude of the impacts will depend on the densities *S. richteri* achieves. Establishment in areas of suboptimal climate will limit dense populations and reduce impacts.
Economic impacts			
2.21. 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	minimal minor **moderate** major massive	**low** medium high	There is no research on impacts of *S. richteri*, principally due to its displacement by *S. invicta* in the USA, and the subsequent problems the latter pest has caused. Various estimates of economic costs due to *S. invicta* in USA have been published, which range from half a

			billion to several billion dollars per year (Pimentel et al. 2000; Morrison et al. 2004). Some more specific accounts exist for regions and impact categories. For example, as cited in CABI (2018): "In 1998, the average household cost for imported fire ant problems per Texas household in urban areas was US $150.79, with US $9.40 spent on medical care. The total annual metroplex (Austin, Dallas, Ft. Worth, Houston and San Antonio) expenditures for medical care costs was 9% or US $47.1 million of the US $526 million total expenditure cost due to *S. invicta* (Lard et al. 2002)". In Australia, the likely impact of *S. invicta* on various economic sectors is estimated at between A$8.5 and A$45 billion (Wylie and Janssen-May 2017). Other regions have made estimations for potential economic costs in case of *S. invicta* invasion. For Hawaii, it was estimated that the impact on various economic sectors would be around US $211 million per year (Gutrich et al. 2007). Economic costs in invaded areas are mainly related to three impact categories: -<u>Impact on agriculture</u>: *S. invicta* can directly damage crops such as corn, sorghum, okra, potatoes and sunflowers by feeding on the seeds, seedlings and developing fruit (Stewart and Vinson 1991; CABI 2018). The impact may also be indirect through the tending of homopteran pests (aphids, scale insects, etc.), which they protect against natural enemies to collect honeydew. However, it must be noted that *S. invicta* also preys on plant pests and may provide benefits to crops.

			The ant also affects livestock by stinging particularly very young, old or confined animals. The ants move to moist areas of the body (eyes, genitals), the yolk of hatching birds and wounds, and begin stinging when disturbed. The stings result in injury such as blindness, and swelling or can even lead to death (CABI 2018). Finally, the ant can also affect the agriculture sector by stinging workers in the field and affecting agricultural equipment (see below). -<u>Health impacts</u>: *S. invicta* can sting people and may cause an allergic reaction that requires medical care and, sometimes, causes anaphylaxis. See social impact below for a description of the medical issue in south-eastern USA. -<u>Impacts on infrastructure and equipment</u>: Ants and their mounds damage roads and electrical equipment. Also domestic electrical equipment may be damaged such as computers, swimming pool pumps, cars or washing machines. Colonies move into buildings or vehicles seeking favourable nesting sites, particularly during flooding and very hot, dry conditions. Fire ant foraging and nesting activities can result in the failure of many types of mechanical (such as hay harvesting machinery and sprinkler systems) and electrical equipment (including air conditioner units and traffic box switching mechanisms) (Wylie and Janssen-May 2017; CABI 2018).
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)?	minimal minor moderate major	low medium high	N/A. Because the species is not present in Europe, there is no current cost of damage.

*i.e. excluding costs of management	massive		
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	minimal minor **moderate** major massive	**low** medium high	It is likely that, if *S. richteri* finds suitable habitats and climates for its development in the Atlantic and continental regions, the impact may be locally major and similar to the impacts of *S. invicta*. But its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability. The magnitude of the impacts will depend on the densities *S. richteri* achieves. Establishment in areas of suboptimal climate will limit dense populations and reduce impacts. In the risk assessment for the Netherlands, Noordijk (2010) also mentions potential ‘indirect’ effects caused by probable import restrictions if fire ants become established indoors in the Netherlands. Many countries, including the countries in the Mediterranean region, are susceptible to fire ant establishments. These countries will have strict regulations on imports of certain goods from infested countries. If the Netherlands harbours fire ants, this will have serious consequences on plant (material) export trade in Europe and worldwide.
2.24. 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)?	minimal minor moderate major massive	low medium high	N/A. Because the species is not present in Europe, there is no current cost of damage.
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	minimal minor **moderate** major massive	**low** medium high	It is likely that, if *S. richteri* finds suitable habitats and climates for its development in the Atlantic and continental regions, the economic costs associated with its management may be locally major and similar to the economic costs of *S. invicta*. But its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability. The magnitude of the impacts will depend on the densities *S. richteri* achieves.

			Establishment in areas of suboptimal climate will limit dense populations and reduce impacts.
Social and human health impacts			
2.26. 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).	minimal minor moderate **major** massive	**low** medium high	*Solenopsis richteri* is a social nuisance in infested areas much like *S. invicta*. Colonies are common around urban areas and are considered an urban pest. Ants also enter buildings and can destroy various domestic equipment. This ant has a painful sting that may cause injury to humans and domestic animals. The sting may produce an immediate, intense pain followed by red swelling. *Solenopsis invicta* significantly affects human health. In south-eastern USA, an estimated 14 million people are stung annually (CABI 2018). A survey in Texas showed that 79% of inhabitants have been stung by the ant in the year of the survey (Drees 2000). While, for most people, the effect of stings is relatively minor, albeit painful, some people are hypersensitive to a protein contained in the venom and, for them, a sting can lead to an anaphylactic shock. Anaphylaxis occurs in 0.6 to 6% of persons who are stung and can be lethal. Several deaths are reported each year in south-eastern USA (DeShazo et al. 1999). A survey in South Carolina showed that 0.94% of the people seek medical attention for *S. invicta* stings and 0.02% are treated for anaphylaxis (Caldwell et al. 1999).
2.27. 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.	minimal minor moderate **major** massive	**low** medium high	It is likely that, if *S. richteri* finds suitable habitats and climates for its development in the Atlantic and continental regions, the impact on social and human health may be locally major and similar to the impacts of *S. invicta*. But its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability. The magnitude of the impacts will depend on

			the densities *S. richteri* achieves. Establishment in areas of suboptimal climate will limit dense populations and reduce impacts.
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	**NA** minimal minor moderate major massive	low medium high	*Solenopsis richteri* is not known for being used as food or feed, being a host or vector of other damaging organisms.
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	**NA** minimal minor moderate major massive	low medium high	No other impacts were found.
2.30. 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?	minimal minor **moderate** major massive	low **medium** high	There are no specific natural enemies of *Solenopsis* spp. in Europe. Thus, only generalist natural enemies of ants may affect the ant and these are highly unlikely to regulate (control) populations.

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ANNEXES

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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*

Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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 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)

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

[10] Not to be confused with „no impact“.

	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[11]	**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.*

[11] 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.

	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** 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

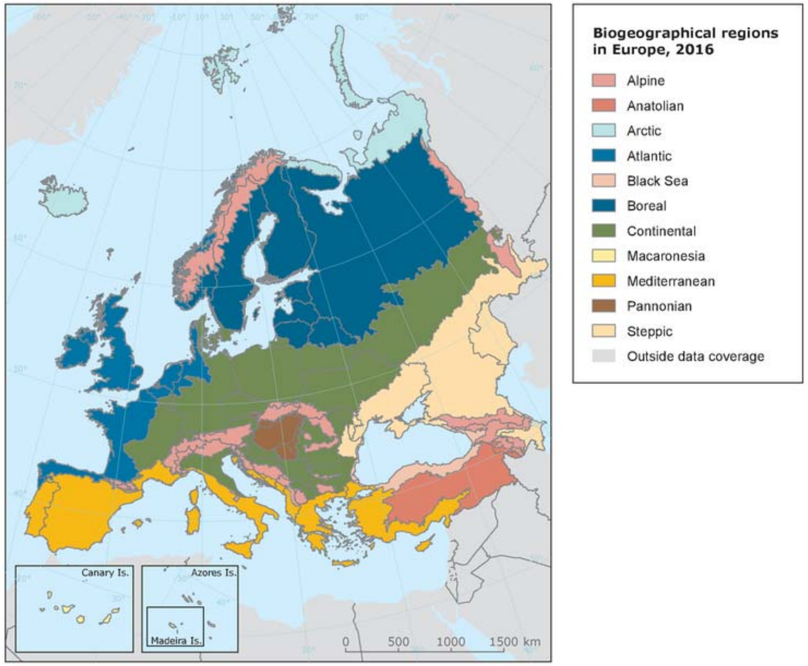
			Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific 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.*

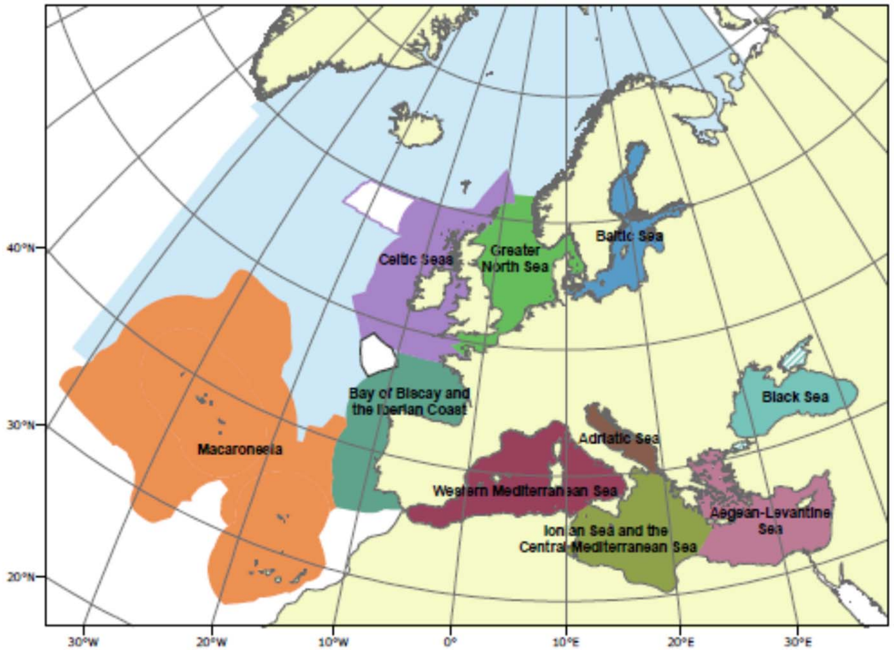
ANNEX V EU Biogeographical Regions and MSFD Subregions

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

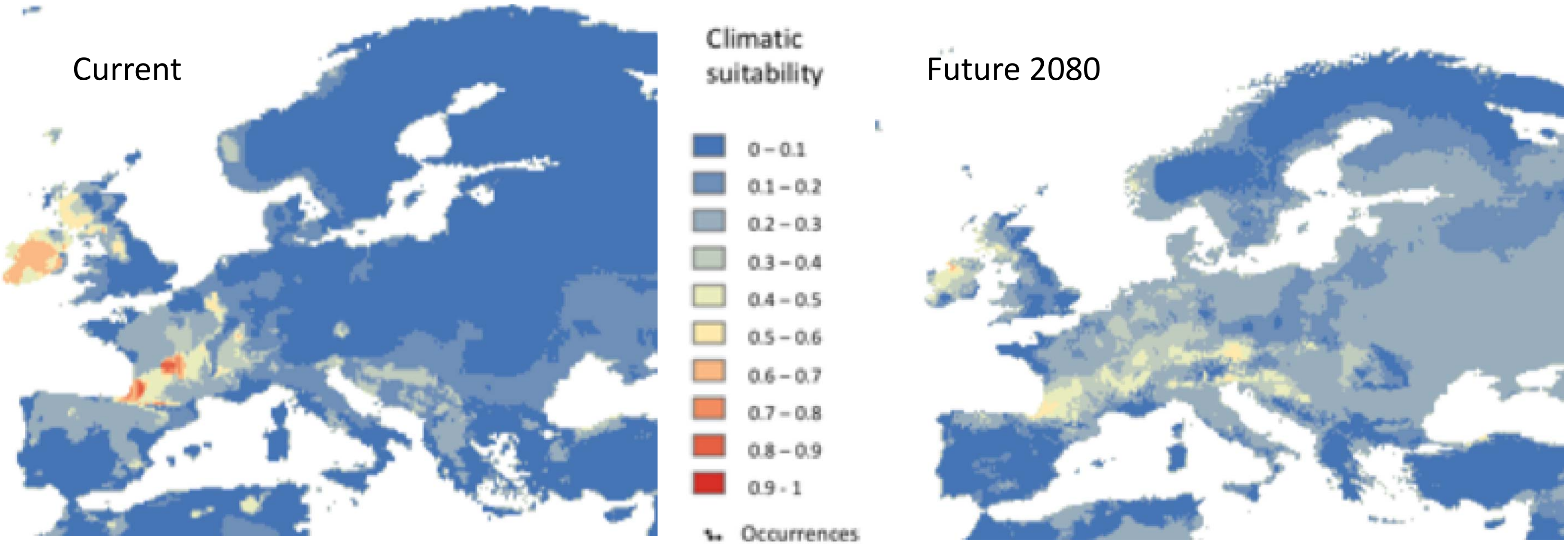
and

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





Species distribution models under current and future climatic conditions (Bertelsmeier et al 2015).



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ii In a scale of low / medium / high, see Annex III

Template for Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Solenopsis richteri***
Species (common name)	**Black Imported Fire Ant**
Author(s)	**Olivier Blight**
Date Completed	**10/18/2018**
Reviewer	**P.Robertson, R.Shaw**

Summary

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 USA and China 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.

There is probably no single method that will allow, alone, the control of *S. richteri* if this latter is introduced in Europe. However; currently the most effective control methods use chemical insecticides. Eradication of single nests in buildings, contained environments and containers is fairly straightforward and can be achieved at low cost. In areas where the climate is suitable for outdoor survival, efforts should be made to eradicate the nest(s) before queens escape into the wild. If *S. richteri* is already established and has begun to spread when first detected, management plans that consist of several applications of chemical insecticides per year over three to four consecutive years, followed by at least two years of intensive surveillance have to be adopted. Countries should have lists of chemical and biochemical insecticides authorised against invasive ants (as bait or contact) ready for use in case an invasion is detected. Chemical control is best when integrated into an IPM system that will reduce the volume needed. Research on biological control should be develop and may constitute a good complement to chemical control.

The management of invasive ants and particularly of S. *richteri* suffers from a lack of operational management experience. This lack of experience with this species increases the uncertainty when defining the most cost-effective measures.

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	**Inspection of imported goods and containers and destruction of nests and ants found at inspection.** 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 impossible, the selection of goods to inspect should consider their nature but also 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 *S. richteri* is present. However, invasive ants do not only arrive from area of origin of the species but also via other localities	To reduce the chances of establishment of exotic ants in Europe, it is necessary to prevent their accidental entry. At the global scale, the number of introduced species in temperate regions is considered to be three and half times higher than the number so far detected (Miravete et al 2014), which highlights the need to set up a common detection method at ports and airports at a European scale. Quarantine inspections and treatments methods used in the USA and China could be adopted in Europe. Similar guidelines as those from USDA (2010, 2015) should be developed for invasive ants in general. 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 ants intercepted at inspections. However, 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 materials actually being inspected. An increased investment in manpower for inspection is needed, combined with a more risk-based approach to better target high risk items. To increase the efficiency of prevention efforts, a careful inspection of goods at port-of-exit should be combined with an active prevention mechanism at ports-of-entry to prevent contamination. New Zealand is likely the most proactive jurisdiction preventing exotic species incursions; their biosecurity activities extending into four ports in three	Medium

	(Bertelsmeier et al 2018) (see Wetterer 2010 for the species introduced range). In addition, in some cases, the species travels in goods and containers that transit via non-infested regions (Ma et al. (2010) in Wang et al. (2013)). To increase efficiency in methods to achieve prevention, a careful inspection of goods at port-of-exit should be associated to an active prevention at ports-of-entry. *Solenopisis richteri* may not be easily recognised by inspectors but all ant species, in particular queens and nests, should be destroyed immediately. USDA (2010) and USDA (2015) provide guidelines on how to treat infested commodities at ports of entry. This can involve immersion or dip treatment, drench treatment, topical treatment, Incorporation of granular insecticides into potting media, etc. Besides visual inspection, baiting is a labour-efficient method of fire ant detection in China, but different techniques are required for specific goods (Hwang et al. 2009; Wang et al. 2013). In addition, the use of sniffing dogs is possible and might be a labour- and cost-efficient method of fire ant detection (e.g. Lin et al. 2011).	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; inspection actions have been reduced by 850 hours per annum, 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 *S. richteri*.	

Methods to achieve eradication	**Mechanical control.** When single nests are found in a confined container, e.g. a flower pot, destruction can be done manually through heat or freezing treatments (USDA 2010, 2015). However, to eradicate outdoor, established populations chemical insecticides are the only effective method for use.	Mechanical control methods are clearly effective when dealing with small number of animals or individual nests, but are likely to be of limited use when dealing with more widespread populations in more open environments. No data are available on costs and effectiveness of non-chemical eradication attempts.	High
	Chemical control with insecticides. Eradication of single nests and, in particular, multiple nests, is best achieved using insecticides. Methods to kill single nests in containers such as potted plants, grass sod, baled hay, etc. are described in USDA (2015). They include immersion or dip treatment, drench treatment, topical treatment, and incorporation of granular insecticides into potting media. Single nests in buildings can also be destroyed using insecticide baits such as those commonly used to combat ants in buildings (Noordijk 2010). Eradication of established population outdoors is more problematic, especially when high numbers of nests are involved. The use of broadcast granular bait-formulated products is recommended. A list of eradication programmes carried out against *S. invicta* outdoors is provided in the GERDA database (Kean et al. 2017), which also lists techniques	Eradication of single nests in buildings, contained environments and containers is rather straightforward and can be achieved at low cost. In areas where the climate is suitable for outdoor survival, efforts should be made to eradicate the nest(s) before queens escape in the wild. There is a lack of specific information on the food preferences and control of *S. richteri*. No differentiation between the two species *S. richteri* and *S. invicta* is made in USA management of fire ants. Many of the commercial ant baits are labelled for use on fire ants in general. Without experimental testing of bait preference and efficacy, the assumption is that control of *S. richteri* using toxic baits should be based on those used for effective control of *S. invicta*. Results of outdoor eradication programmes targeting *S. invicta* have been variable (see Kean et al. 2017 who describe 12 eradication programmes). The ant has been eradicated from various areas, including from climatically suitable ones (e.g. New Zealand and parts of Australia and Taiwan), but many eradication attempts failed (in various areas in USA, Australia and China). The eradication plan that has been put in place in Australia in early 2000s has cost so far about AUS$300 million for treating an area of nearly 70,000 ha. It has achieved the eradication of at least two incursions but others have not yet been eradicated, although at least one of them is now under containment (Invasive Species Council 2015). The primary reasons for the failure of eradication attempts in USA include: (i) the inability to attain absolute (100%) control using available	High

	and products used for the eradication and references. Considering the similarity (phylogeny, biology and ecology) between *S. richteri* and *S. invicta*, examples of success and failures in attempts to eradicate *S. invicta* are relevant to *S. richteri*.	products; (ii) the large area of infestation; (iii) high cost of treatment; (iv) inability to uniformly treat an entire area of infestation (Drees et al. 2006); and (v) the ability of fire ants to rapidly spread even before eradication efforts are put in place (Drees et al 2013). In Taiwan, an infestation of 13 Ha with a total of 1,578 mounds was successfully eradicated within one year. However, eradication programmes that were most successful were those involving one or a small number of nests, such as the two successes achieved in New Zealand (Christian 2009). The eradication of *S. invicta* in early 2000s in Auckland covered less than 1 Ha but cost NZ$1.4 million. In Australia, an eradication programme of *S. invicta* was evaluated at AU$200 million (Hoffmann et al 2010). This programme had noteworthy success, and highlighted valuable lessons. For example, the programme revealed clear differences in the efficacy of bait application; aerial application has proven to be the most efficient strategy followed by hand and land vehicles application methods. A key component to the success of the eradication program conducted in Australia was that it was adequately though and modestly funded to its conclusion (approximatively AU$60 000 for 3ha treated) with good cooperation between the numerous stakeholders within the area targeted. This is the only successful documented attempt to eradicate *S. geminata*. It is of utmost importance to start eradication programmes as soon as possible. Therefore, it is essential to develop contingency plans against this and other invasive ants at the European scale to be ready when ant establishments are notified. These plans should include considerations on social and environmental issues related to the use of chemical controls as well as lists of products licensed for ant control indoors and outdoors.	
Methods to achieve management	**Chemical control.** Chemical control will target not only the worker but also, and importantly, the queen, to kill nests.	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	High

	Options include broadcast granular bait-formulated products, treatment of individual ant colonies in mounds and surface or barrier treatments using contact insecticides (Drees and Gold 2003; Drees et al. 2013; CABI 2017). Common insecticides that can be used for fire ant control in USA are provided by Drees et al. (2013) and Greenberg and Kabashima (2013). Different insecticides will be used for the different options. Drees et al. (2013) also discuss the limitations of chemical treatments and their integration into an IPM system. Wang et al. (2013) reviews research in China on chemical and other control methods *against S. invicta*. Of interest is the effective use of bioinsecticides (e.g. spinosad and plant extracts) and the good results obtained using the two-steps approach, i.e. first a bait is broadcasted over large areas and, then, remnants of ant mounds are treated individually with contact insecticides.	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. Data on the management costs of *S. invicta* using insecticides in USA are available (Barr et al. 2005). Conventional bait insecticides cost approximately US$10 per 0.4 ha for broadcast application, and with the cost of application, total treatment costs of 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).	
	Cultural and sanitary methods. Cultural management methods in cattle production have been developed to limit damage of *S. invicta* to livestock. For example, the use of disc-type cutters, the quick removal of hay bales from the field and the scheduling of cow fertility programmes to avoid calving during hot, dry summer months (Drees et al. 2013; CABI 2017). Other	These and other cultural and sanitary methods could be considered for use in Europe. There is no information on the cost-effectiveness of these methods.	Medium

	approaches can be used in different environments. In particular, it has been shown that frequent disturbance of mounds causes colonies to move to less disturbed areas (Drees et al. 2013). More generally, hindering favourable habitats may be considered. Disturbed areas should be allowed to regenerate after which the vegetation succession will make the site less suitable for *S. invicta* (Noordijk 2010).		
	Biological control. Many natural enemies of *S. richteri* have been identified (Briano et al 2012). They occur in its native range and are believed to keep populations at lower densities than in the invaded regions. Many of them have been studied for their potential as classical biological control agents. Several parasitic flies of the genus *Pseudacteon* (Diptera: Phoridae) have been introduced and have established and spread in the USA since 1997 (Graham et al., 2003; Williams et al. 2003; Morrison 2012). *Pseudacteon tricuspis* and *Pseudacteon curvatus* attack the Black imported fire ant where it occurs in the USA, but their impacts on colony survival remain unknown (Callcott et al. 2010). In addition, microsporidia and viruses have been studied and at least a microsporidia, *Kneallhazia solenopsae*, has been found infecting *S. invicta* and	*Pseudacteon* spp. and the pathogens could possibly be considered for introduced to Europe since they are specific to one or a few exotic *Solenopsis* spp. and should therefore have limited side effects on the environment, with maybe the exception of native *Solenopsis spp* (Folgarait et al. 2002; Oi and Valles 2012). However, so far, the effect of *Pseudacteon* spp. on *S. invicta* population densities has not been demonstrated, possibly because average parasitism rate per colony is too low (Morrison and Porter 2005; Tschinkel 2006; Morrison 2012). Some data on the cost of releasing *Pseudacteon* spp. in the USA are available in Drees et al. (2013) but they are not really applicable to Europe since they do not include the necessary significant pre-release investigations. The production cost of *Pseudacteon* is estimated at $1.00 per fly. Five thousand flies were released near Gainesville (Florida) in 1997. By fall of 2005, they spread to over 90 000 square kilometres (Drees et al. 2013). The cost of this release was estimated at $10 000, but considering the spread of the species, treatment cost estimate dropped down to at $0.0001/ha. Classical biological control, i.e. the introduction of exotic natural enemies for their permanent establishment and long-term control of a pest, is a very cost-effective method since no action is required after releases and establishment. However, the level of control that would be achieved through the release of the flies is very uncertain.	Medium

	S. richteri established in North America (CABI 2017). The entomopathogenic fungus *Beauveria bassiana sensu lato* has also been applied against *S. invicta* in the field in established mounds (Bextine and Thorvilson 2002). Whilst the efficacy of this method has been demonstrated under laboratory conditions, few studies have validated its use in the field. However, Bextine and Thorvilson (2002) conducted two field experiments, one at the mounds scale and another at a site scale (700m^2). In both experiments they successfully inactivated up to 80% of the mounds. Similarly, Kalfe et al. (2010) succeed in inactivating 70% (22 treated) of the mounds treated with *B. bassiana*. In both studies, the most efficient delivery form was the use of baits (e.g. fungal pellets coated with peanut oil) instead of a direct application of the fungus.	In the Southern USA, it is thought that, collectively, these natural enemies may help reducing the frequency of insecticide applications required to maintain *S. invicta* control, but are not sufficiently effective to achieve control on their own (Oi et al. 2007; Drees et al 2013). The different agents also affect the species in different ways which influences their possible effectiveness. *Pseudacteon* spp. parasitize a small percentage of workers but indirectly affect colonies by suppressing daytime foraging behaviours whereas disease organisms directly affect ants and colony health (Drees et al. 2013).	
	Integrated pest management. To keep population levels below those that cause economic, social, or ecological damage, the integration of chemical, cultural, biological and regulatory methods into an IPM system is needed (Hoffmann et al 2010). Drees et al. (2013) provide the latest information on IPM methods developed against *S. invicta* in the Southern USA. IPM design considerations include management goal(s), action level(s), ant form	There is probably no single method that will allow, alone, the control of *S. richteri* if this latter is introduced in Europe. The long expertise gained in USA on the development of IPM programmes against *S. invicta*, reviewed and analysed in Drees and Gold (2003) and Drees et al. (2013) will undoubtedly help developing specific programmes for Europe. Their cost is impossible to assess in the present situation.	High

	(monogyne or polygyne), presence of nontarget ant species, size of treatment area, seasonality, implementation cost, and environmental impact are also presented. Their conclusion is that "There is no single best IPM program for imported fire ants. Programs designed and implemented using IPM concepts will vary due to multiple factors including the presence and abundance of fire ants and other ant species, together with the level and seasonality of control desired, established natural enemies in the management area, availability of registered insecticide products for the use sites involved, environmental concerns, and cost of application(s) that include time and labour. Optimally, elegant IPM programs would be target specific, threshold driven, environmentally friendly and cost-effective. With eradication unlikely to succeed in areas larger than isolated spot infestations, containment and suppression become the overriding goals. However, within such larger landscapes, maximum control is attainable using well-designed treatment programs that, where justified, periodically use selected chemical methods."		

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Name of organism: *Solenopsis geminata,* Fabricius 1804.

S. geminata worker (major), credits : Alex Wild

Author(s) 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: Wolfgang Rabitsch, Environment Agency Austria, Vienna, Austria

Peer review 2: Jørgen Eilenberg, University of Copenhagen, Denmark
Peer review 3: Richard Shaw, CABI, UK
Peer review 4: Marc Kenis, CABI, Switzerland

This risk assessment has been peer-reviewed by two independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study.

Date of completion:
10/18/2018

[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

Contents

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**[2]	**COMMENT**
Summarise Entry[3]	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	The most important pathway of introduction for *S. geminata* to Europe is the unintentional translocation of nests as contaminant of nursery material (including soil) and as stowaway/hitchhiker in container/bulk or other commodities (e.g. vehicles, machinery, packaging material). However, the propagule pressure of nests is largely unknown. Queen ants are also likely to arrive as hitchhikers, but only aircraft will allow a transfer fast enough for survival.
Summarise Establishment[4]	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Based on a global species distribution model, *S. geminata* could become established in all countries around the Mediterranean Sea, with both the Southern Atlantic Coast from Southern France to Spain and the Adriatic coast of Italy being particularly suitable. 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. This assessment is based on one species distribution model. The use of additional models may improve the prediction and confidence level of this assessment.
Summarise Spread[5]	very slowly slowly **moderately** rapidly	low medium **high**	In all potentially infested biogeographical regions, *S. geminata* will probably spread moderately rapidly compared to other insects. Although *S. geminata* can spread unaided over several kilometres per year, its

[2] In a scale of low / medium / high, see Annex III
[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I
[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I
[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly

	very rapidly		spread will occur mainly through human-assisted transport, in particular with soil and infested items, but its distribution will be constrained by climate, habitat suitability and competition from other dominant ants. It is likely that if established, the ant will have a patchy distribution in Southern Europe, with moderate densities and extent in open and sunny disturbed habitats.
Summarise Impact[6]	minimal minor **moderate** major massive	low **medium** high	The species has a moderate to major environmental, economic and social impact elsewhere in the world. Similar impacts may occur in Southern Europe. However, the transferability of this impact to Europe is hindered by uncertain data on habitat/climatic suitability that may limit the geographic area that is most favourable to the insect. 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[7]	low **moderate** high	low **medium** high	*Solenopsis geminata* is not one of the most damaging invasive ants on earth but probably the most successful one at invading and colonising 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 dominant ant species. It might have environmental, economic and social impact in some areas of Southern Europe, but the extent of its potential distribution remains unclear.

[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

[7] In a scale of low / moderate / high

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established* (future)	Invasive (currently)
Austria	-	-	-	-
Belgium	-	-	-	-
Bulgaria	-	-	-	-
Croatia	-	-	YES	-
Cyprus	YES	-	-	-
Czech Republic	-	-	-	-
Denmark	-	-	-	-
Estonia	-	-	-	-
Finland	-	-	-	-
France	-	-	YES	-
Germany	-	-	-	-
Greece	YES	-	YES	-
Hungary	-	-	-	-
Ireland	-	-	YES	-
Italy	YES	-	YES	-
Latvia	-	-	-	-
Lithuania	-	-	-	-
Luxembourg	-	-	-	-
Malta	-	-	-	-
Netherlands	YES	-	-	-
Poland	-	-	-	-
Portugal	-	-	YES	-
Romania	-	-	-	-
Slovakia	-	-	-	-

Slovenia	-	-	YES	-
Spain	-	-	YES	-
Sweden	-	-	-	-
United Kingdom	YES	-	-	-

*Countries with suitability index >0.5 in foreseeable climate change in Bertelsmeier et al. (2015).

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Alpine		-	-	-
Atlantic	YES	-	YES	-
Black Sea		-	-	-
Boreal		-	-	-
Continental		-	YES	-
Mediterranean	YES	-	YES	-
Pannonian		-	-	-
Steppic		-	-	-

Marine regions and sub-regions of the risk assessment area

	Recorded	Established (currently)	Established (future)	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				

SECTION A – Organism Information and Screening

Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Scientific name: *Solenopsis geminata* Fabricius 1804 Class: Insecta Order: Hymenoptera Family: Formicidae Genus: *Solenopsis* Westwood, 1840 There is one subspecies: *Solenopsis geminata micans* Stitz, 1912 *S. geminata* is a highly polymorphic species, with a wide range of worker size within the colony (head width = 0.55 – 2.30 mm). It shows considerable variation in coloration. *Solenopsis geminata* can occur in a "red form" that is more abundant in open areas and in a "black form" that prefers forested areas (Longino 2005). The environmental or genetic determinants of these forms are unknown. As a result of this variability, combined with some poor taxonomic work, *S. geminata* has been described repeatedly under many different names, now designated as junior synonyms (Wetterer 2010). Synonyms: *Atta geminate* Fabricius, 1804; *Solenopsis geminata rufa* (Jerdon, 1851). A comprehensive and regularly updated list can be found at www.antweb.org. Common name: Tropical Fire ant (TFA)
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 wild, in confinement or associated with a pathway of introduction]	The genus *Solenopsis* contains about 200 species, among which 18 to 20 are “true fire ants”, which all look very similar and have the potential of becoming invasive. Fire ants are a group of related species (*Solenopsis geminata* group) that has its centre of diversity in southern South America. A key for separation of the taxa in the *S. geminata* species-group was provided by Trager (1991).

A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	A risk assessment has been made for fire ants (*Solenopsis* spp.) in the Netherlands, which concludes that, although they are regularly found during import inspections in the Netherlands, it is unlikely that they can establish outdoors in the country (Noordijk 2010). This is particularly true for *Solenopsis geminata* which is more thermophilic than *S. invicta* and *S. richteri*. However, establishment in permanently heated buildings is possible, and can cause nuisance to humans through their sting and the destruction of equipment such as electrical installations (including air conditioner units, computers, etc.) (Noordijk 2010). These conclusions are similar to those in the present risk assessment for the North Atlantic biogeographical region. Another RA has been carried out for New Zealand, which classified *S. geminata* as having a *high risk* of entry but a *low risk* of establishment and spread (Harris 2005). However, RA made for different regions are not easily comparable.
A4. Where is the organism native?	The exact limitation of the native range of *Solenopsis geminata* remains unclear (Gotzek et al. 2015). It is disputed, in part because the species is continuously distributed from the southern United States to northern South America (Holway et al. 2002). Trager (1991) considers *S. geminata* native to the south-eastern coastal plain of Florida to Texas south through Central America to northern South America, including the coastal areas of north-eastern Brazil, west through the Guianas to the Orinoco Basin, the western Amazon Basin and coastal areas of Peru. Wetterer (2011) wrote: "*S. geminata* is originally from the New World tropics and subtropics. However, the extent of the native range of *S. geminata* in the New World remains unclear. *Solenopsis geminata* is almost certainly native to South America, Central America and Mexico, and most authors consider *S. geminata* as native to the South-eastern US." In fact, *S. geminata* in US might be a mix of native and exotic populations (Wetterer 2011). *Solenopsis geminata* is most abundant in open and disturbed sunny areas. It is common in agricultural areas and around human settlements. In the lowlands it is found not only in the open but may also penetrate into forest understory, albeit at lower density (see section A1 above about red and black forms). At higher elevations it is restricted to open areas and does not extend into closed-canopy forest. There is anecdotal evidence that *S. geminata* occurrence in forest understory is increasing, perhaps due to effects of fragmentation (e.g. in New Caledonia, Olivier Blight pers. obs.).
A5. What is the global non-native distribution of the organism outside the risk assessment area?	*S. geminata* has been extraordinarily successful in spreading into five continents and has colonized many tropical islands on all the oceans. In the New World, it has been reported from all South and Central American countries, the Southern US from California to Virginia, and every island group in the West Indies. However, a number of these records were possible misidentifications (e.g. of *Solenopsis xyloni*, *Solenopsis gayi*, *Solenopsis saevissima*) (Wetterer 2010). In the Old World, *S. geminata* is widespread

	through tropical and subtropical Asia, Australia, and Oceania. The documented range of *S. geminata* in Africa is much more limited and many records appear to be a different species (Kouakou et al. 2017).
A6. In which biogeographic region(s) or marine sub-region(s) in the risk assessment area has the species been recorded and where is it established?	<u>Recorded:</u> Mediterranean and Atlantic biogeographic regions. The species was recorded in Italy before 1861 (Mayr 1861 as *D. drewseni*), in England in 1932 (Donisthorpe 1943), in Greece in 1982 and 1988 (Collingwood 1993), in Cyprus before 1997 (Collingwood et al. 1997) and Netherlands in 1992 (Boer and Vierbergen 2008) (see Wetterer 2010). <u>Established:</u> The species currently is not established in the risk assessment area, neither in the wild nor indoors. One population was established in a building in the Netherlands (Atlantic Biogeographic Region) and was eradicated (Noordijk 2010).
A7. In which biogeographic region(s) or marine sub-region(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change?	<u>Current climate (suitability index above 0.5 in Bertelsmeier et al. (2015), see annexe 1):</u> Atlantic, Continental and Mediterranean <u>Future climate (suitability index above 0.5 in Bertelsmeier et al. (2015), see annexe 1):</u> Atlantic, Continental and Mediterranean According to the only available species distribution model (Bertelsmeier et al. 2015), *S. geminata* will not establish widely in Europe under both current and future climatic conditions until 2080. However, it will have the capacity to do so in Atlantic (North of Spain and Portugal, South West coast of France and South East of Ireland), Continental (North of Italy) and Mediterranean (Spain, France, Italy, Croatia, Cyprus, Greece and Malta) Biogeographic Regions. According to the applied models, overlap between species' current and future potential distributions is 98.1 % (Bertelsmeier et al. 2015). For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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.	Recorded in the following Member States: Cyprus, Greece, Italy, Netherlands, United Kingdom (Wetterer 2010) <u>Established:</u> The species currently is not established in the risk assessment area.

	Workers have been found occasionally during import inspections, and in at least one occasion in the Netherlands, a nest has been found in an apartment building (Noordijk 2010). It was eradicated using chloredecone.
A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	Current climate (suitability index above 0.5 in Bertelsmeier et al. (2015): Croatia, Cyprus, France, Greece, Ireland, Italy, Malta, Portugal, Slovenia and Spain. Future climate (suitability index above 0.5 in Bertelsmeier et al. (2015): same countries as above mentioned According to the only available species distribution model (Bertelsmeier et al. 2015), *S. geminata* will not become established widely in Europe under both current and future climatic conditions until 2080. It will have the capacity to establish in Southern Europe: Croatia, Cyprus, France, Greece, Italy, Slovenia and Spain. However even in Southern Europe habitat suitability is currently low and will likely be so in the future except for the northern part of Italy. There are no other published predictions of the current and future potential of *S. geminata* establishment in Europe.
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?	Yes. It is considered to be amongst the most widely distributed invasive species on earth. It has colonized almost all continents and has ecological and economic impacts albeit its impacts are often considered lower than other invasive ants (Holway et al. 2002).
A11. In which biogeographic region(s) or marine sub-region(s) in the risk assessment area has the species shown signs of invasiveness?	None. There was one established population in a building in the Netherlands, but it was eradicated using chloredecone.
A12. In which EU member states has the species shown signs of invasiveness?	None. There was one established population in a building in the Netherlands, but it was eradicated using chloredecone.
A13. Describe any known socio-economic benefits of the organism.	At present there are no socio-economic benefits in regions where the species is invasive. The species is not present in the RA area.

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."
- The classification of pathways developed by the Convention of Biological Diversity shall be used for detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism?	none very few few moderate number **many**	low medium **high**	*S. geminata* has been intercepted from a variety of commodities (ornamental plants and fruits) and origins (South America, US) at US ports and airports since 1910 (Blight et al. unpublished data). *S. geminata* intercepted

[8] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf
[9] 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 respond N/A and move to the Establishment section)	very many		in the Netherlands originated mainly from Thailand (Noordijk 2010).
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	a) Transport-Stowaway (Hitchhikers in or on airplane) 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.)		*Solenopsis geminata* is termed a “tramp” ant, it can hitchhike with many commodities through many pathways. However, only the entry of queen ants and nests present a risk of establishment. In the case of an independent colony foundation, the queen has to find a suitable place quickly after the nuptial flight. These restrictions limit the number of active pathways as the risk of predation is very high. Harris (2005) provided a very detailed analysis of potential pathways of introduction of *S. geminata* in New Zealand, which is also highly relevant for Europe. Noordijk (2010) provides a brief assessment of pathways for the Netherlands as well as interception data.
Pathway name:	a) Transport-Stowaway (Hitchhikers in or on airplane)		
1.3a. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional **unintentional**	low medium **high**	This concerns only new mated queens.

1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Sub-note: In your comment 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.	very unlikely unlikely **moderately likely** likely very likely	**low** medium high	Although many individuals may travel this pathway, new colonies are established by solitary fertile queens following a mating flight. Queens seek moist areas within a few kilometres of the parent colony. Once a suitable site is found the female sheds her wings and digs a small burrow into the soil and seals it. Although few data is available on ant interceptions at ports and airports, the proportion of queens in interception database is very low which suggests a relatively low number of newly-mated queens travelling along this pathway.
1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	Ant queens are able to survive several tens of days using their own reserves before the first workers emerge. However, likelihood of survival will decrease with increasing travel duration, but is possible. Multiplication and the establishment of a small nest during such an intercontinental flight however is highly unlikely.
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	N/A. There are no management practices against hitchhiking ants or ant queens in or on airplanes in place.
1.7a. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Detection rates for solitary queens or even several queens or small nests are low; in general, ants are not easy to detect in cargo airplanes and detection rate thus will be low.
1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	During warm months winged individuals are found in large numbers in mature colonies. Reproduction of ant queens can occur over several months and commodities with which ants can enter Europe occur throughout the year. However, among the 21 records between 1984 and

			2010 in the Netherlands no *S. geminata* queen has been intercepted.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Many airports in the Mediterranean region are surrounded by suitable habitats including irrigated/watered gardens and parks. Indeed, this species simply requires soil as a substrate in which to establish a nest and has been found to occur in diverse degraded habitats particularly in warm opened habitat.
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely **moderately likely** likely very likely	**low** medium high	The likelihood is scored moderately likely because the number of queen ants travelling through this pathway is expected to be relatively low and the duration of the transportation would not favour the survival of the queen.
Pathway name:	*b)* Transport-Contaminant (nursery material and other matters from the horticultural trade)		
1.3b. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	This concerns both fully developed nests (with active workers) and newly-founded nests (before workers are developed and start foraging) transported in nursery material by the horticultural trade. Newly-founded nests can also be formed by queens transported in ships before the nursery material arrives at destination.
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Sub-note: In your comment 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.	very unlikely unlikely moderately likely **likely** very likely	**low** medium high	There are very limited data on ant nests arriving through the horticultural trade in Europe. At least some nests have reached Europe (the Netherlands), New Zealand, Australia and US. 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 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. Flower pots are one of the preferred habitats

			for *S. geminata* 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 harbour ant nests. Monogyne and polygyne forms occur. Polygynous forms are mainly found in the introduced range of *S. geminate* and may originate via a founder event from a local monogyne population (Ross et al. 2003). The number of workers in a polygynous nest can vary enormously, from 4 000 to hundreds of thousands (Taber 2000). Way et al. (1998) estimated up to 100 000 *S. geminata* workers in a large nest and at least 500 000 in 100 metres of rice field edge. Ant nests might get onto the pathway in large numbers as contaminant of horticultural materials contains soil.
1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Ant queens are able to survive a few weeks using their own reserves before the first workers emerge. However, likelihood of survival is high but nevertheless will decrease with increasing travel duration. Multiplication of a small nest during intercontinental translocation however is highly unlikely.
1.6b How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely **likely** very likely	**low** medium high	Horticulture plants and soils/substrates are usually chemically treated before shipment but can be infested after treatment either before departure or during transport.
1.7b. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely**	low **medium** high	Fully developed nests are quite visible. Newly-founded nests with few queen(s) and workers in the soil/substrate can easily arrive undetected.

	very likely		
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	The horticultural trade is active throughout the year.
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	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
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	We consider this pathway as the most likely pathway of entry of *S. geminata* into Europe. Noordijk (2010) also considers the horticultural trade as the most likely pathway for introduction in the Netherlands.
Pathway name:	c) Transport-Stowaway (nests transported in container/bulk, including sea freight, airfreight, train, etc.)		
1.3c. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	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 and fully developed nests. 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.
1.4c. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year?	very unlikely unlikely moderately likely **likely** very likely	**low** medium high	There are very limited data on ant nests arriving in Europe. Sea containers and all articles of commerce cited above were scored by Harris (2005) as presenting a high likelihood of introduction for nests of *S. geminata*.

Sub-note: In your comment 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.			The number of workers in a polygynous nest can vary enormously, from 4000 to hundreds of thousands (Taber 2000). Way et al. (1998) estimated up to 100 000 *S. geminata* workers in a large nest and at least 500 000 in 100 metres of rice field edge. Ant nests might get onto the pathway in large numbers as stowaway in containers or other bulk freight, including soil. The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.
1.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Queens in ants are able to survive a few weeks using their own reserves before the first workers emerged. However, likelihood of survival is high but nevertheless will decrease with increasing travel duration. Multiplication of a small nest during intercontinental translocation however is highly unlikely.
1.6c How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	In most of the commodities in this pathway, there are no management practices in place.
1.7c. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Many of these commodities are not carefully inspected. While established nests are usually obvious, newly-founded nests are often inconspicuous. Newly-founded nests with few queen(s) and workers could easily arrive undetected.
1.8c. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely **likely**	low medium **high**	Commodities that can carry *S. geminata* are introduced to the risk assessment area throughout the year.

	very likely		
1.9c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Several of the potential commodities and items in which nests can hide can be transported to suitable habitats since the ant particularly likes disturbed soils, which are found everywhere, specifically in urban and semi-urban habitats.
1.10c. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Given the high numbers and types of containers, commodities and items that can be associated with *S. geminata*, this pathway can be considered as having a high likelihood of entry, as determined by Harris (2005) and Noordijk (2010). Sixteen of the 46 interceptions of *S. geminata* in Australia were in containers including empty ones (Source: Department of Agriculture, Fisheries and Forestry, Canberra).
End of pathway assessment, repeat as necessary			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	very unlikely unlikely moderately likely **likely** very likely	low medium **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 freights.
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Climate change is not changing the risk of introduction or likelihood of entry based on the mentioned active pathways.

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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Bertelsmeier et al. (2015), using a climate matching model (Maxent) based on present distributions, mapped suitable areas globally for 15 of the worst invasive ant species (incl. *S. geminata*). They showed that less than 2% of the European continent is presently suitable for *S. geminata*,
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Other abiotic conditions should not be a constraint on the establishment of *S. geminata* in Europe, except for high-altitude environments. The ant particularly likes open disturbed soils, which are found everywhere, specifically in urban and semi-urban habitats (Perfecto and Vander Meer 2011).
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	very isolated isolated **moderately widespread** widespread ubiquitous	low **medium** high	*Solenopsis geminata* prefers open disturbed habitats, which are found everywhere in Europe. However, as a tropical species it needs hot temperatures to complete its life cycle (Cokendolpher and Francke 1985; Braulick et al. 1988), which may limit its distribution to the Mediterranean region, at least in natural areas. There is no experimental data on cold climate tolerances of *S. geminata*. However, preferred temperatures for brood development are reported to be above 22°C.

1.16. 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?	**NA** very unlikely unlikely moderately likely likely very likely	low medium **high**	*Solenopsis geminata* does not require another species for establishment.
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	*Solenopsis geminata* is an ecologically dominant ant in disturbed ecosystems and open habitat within its native range (Morrison 2000). There is probably intense competition with other dominant species in some habitats. However, *S. geminata* does not appear to be highly competitive compared to other invasive ant species. It has been replaced by *S. invicta* in many places in US (Tschinkel 1988). 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 colonies of many hundred thousands of individuals. The Argentine ant was superior to the highly competitive *S. invicta* during asymmetrical confrontation tests (numerical advantage for the Argentine ant) under laboratory confrontations (Kabashima et al 2007). The Argentine ant is largely distributed along the Mediterranean coast from Portugal to Italy through Spain and France. It has been also recorded in Malta and Greece. Nonetheless, where these competitive species are not present the establishment may easily occur.

			Moreover, these species have a more temperate distribution and may have a competitive advantage over *S. geminata* in the risk assessment area. Nonetheless, where these competitive species are not present then establishment could easily occur.
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	Only few *Solenopsis* spp. are native to Europe, and no specialist natural enemies of *Solenopsis* spp. 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.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	No specific management practices are in place against invasive ants in the wild in Europe. Eradication of single nests is straightforward in buildings (e.g. Noordijk 2010) but much less so outdoors. However, some eradication programmes have succeeded at a local scale, such as in Australia (Hoffmann and O'Connor 2004).
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	**very unlikely** unlikely moderately likely likely very likely	low medium **high**	There have been no management practices applied in the risk assessment area but conventional management practices to date should not facilitate establishment.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	The eradication of *S. geminata* outdoors is difficult, especially when populations reach high densities of nests and individuals. However incipient colonies can be successfully eradicated (Hoffmann et al. 2016).
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	*Solenopsis geminata* has single queen (monogynous) and multi-queen (polygynous) populations. Polygynous forms are mainly found in the introduced range of *S. geminata*.

			The polygynous form can more easily establish because the higher number of queens increases reproduction potential, especially in the critical early stages of establishment. The number of workers in a polygynous nest can vary enormously, from 4000 to hundreds of thousands (Taber 2000). Way et al. (1998) estimated up to 100 000 *S. geminata* workers in a large nest and at least 500 000 in 100 metres of rice field edge. Few data are available on the biology of *S. geminata*. The queen lay around 10 to 15 eggs each day for up to 10 days after which she will stop laying eggs until the workers are mature (source: iss.org). On an indicative basis, inseminated females (queens) of *Solenopsis invicta* lay up to 200 eggs per hour (Tschinkel 1988). Within one year, the colony can grow to several thousands of workers, within three years it can reach up to 230,000 workers (Tschinkel 1988). The peculiar, almost unique, reproductive caste system of these eusocial insects can facilitate establishment. 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).
1.23. How likely is the adaptability of the organism to facilitate its establishment?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	*Solenopsis geminata* is probably the most widespread invasive ant, highlighting its capacity to adaptation when introduced to new environments. However, several factors can constrain establishment of this species. Despite *S. geminata* being a generalist, opportunistic species, it requires

			open, sunny places, and favours those that are associated with humans. Also, in contrast to the invasive *S. invicta*, it has a restricted flight period. Nuptial flights have been recorded only during the warmest seasons. Similarly, foraging and brood development are restricted by cold temperatures. Foraging was not recorded below 15°C (Wuellner and Saunders 2003). In Australia, *S. geminata* is assigned to the hot climate specialist functional group (Andersen and Reichel 1994).
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Most invasive ants, which are among the most invasive insects worldwide, establish following the entry of single nests or queens (Holway et al. 2002). In the case of *S. geminata*, it may increase its success of establishment as low genetic diversity is associated with the polygynous form of colonies. Therefore, low genetic diversity does not seem to be a barrier to establishment.
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	*Solenopsis geminata* may be the most widely distributed invasive ant (Wetterer 2010a) which highlight its capacity to establish outside its native range. However, considering climatic requirements and potential competition with other dominant ants, *S. geminata* is moderately likely to establish in Europe.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Sub-note: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	As shown with interception data from countries such as the Netherlands (Noordijk 2010), US (Bertelsmeier et al. 2018), New Zealand (Harris 2005), *S. geminata* and related *Solenopsis* spp. are regularly intercepted at ports of entry. However, in most cases, these are sterile workers that cannot

			establish in the wild. Ants are not listed as quarantine pests in the EU and, therefore, interception data are not good indicators of their frequency of entry because they do not have to be mentioned in the national and international lists of intercepted pests. It has to be assumed that there is a considerable number of unreported cases.
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	very unlikely unlikely **moderately likely** likely very likely	**low** medium high	In the Mediterranean biogeographical region, establishment under current conditions is likely at least in the most open and hot habitats. Also, both the southern Atlantic (Southern France, Northeast of Spain and North of Portugal) in the Mediterranean region and parts of the Continental (Northeast of Italy and Slovenia) region are considered to be potentially susceptible (Bertelsmeier et al. 2015). However, all these areas are restricted and cover a very limited area. The absence of other, more regional, models predicting *S. geminata*'s possible distribution in Europe limits our conclusions. The question is also scored "moderately likely" because considering the great invasion success of *S. geminata* throughout the world for 150 years, the absence of established populations in Europe so far suggests that abiotic and/or biotic filters constrain its establishment under current climatic conditions.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Under foreseeable climate change, *S. geminata* may establish in the Atlantic, Mediterranean and Continental biogeographic regions (according to Bertelsmeier et al. 2015). The overall area suitable for *S. geminata* will not significantly increase in the future. However, some of the current suitable

			areas such as in Italy and Slovenia are predicted to be more suitable. To consider a range of possible future climates, Bertelsmeier et al. (2015) 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. The absence of other, more regional, models predicting *S. geminata*'s possible distribution in Europe limits our conclusions.

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	minimal minor **moderate** major massive	low medium **high**	New colonies are founded by winged females, capable of flying long distances. This allows new colonisations a long distance from the source population (Holway et al. 2002). Nuptial flights will result in rapid spread outwards from a site of establishment. Newly mated queens of *S. geminata* seek moist areas, normally within 2 km of the mother colony. Polygynous colonies can also spread by "budding", i.e. queens disperse only short distances over land and take workers with her to start a new colony. However, this type of colony foundation has not been observed in *S. geminata*. Such a strategy would not allow a rapid spread but increase nests densities by increasing survival rates of queens and colonies. The question is scored "moderate" because it is likely to spread more slowly by natural means than by human assistance.
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the	minimal minor moderate	low medium **high**	Human assisted pathways of spread are the agricultural and horticultural trade of plants, plant

mechanisms for human-assisted spread) and provide a description of the associated commodities.	**major** massive		materials, and soil/substrate as well as other movements of commodities.
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	a) Transport-Contaminant (Contaminant nursery material) b) Transport-Stowaway (Container/bulk, including road transport, sea freight, airfreight, train, etc.) c) Unaided (Natural dispersal)		
Pathway name:	a) Transport-Contaminant (Contaminant nursery material)		
2.3a. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	
2.4a. 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?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Within Europe, movements of potted plants are unrestricted. Soil/substrate in potted plants is a favourite media for nesting (see entry section 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 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 contaminant of horticultural materials including soil.

			The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.
2.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Ant queens that independently found new colonies are able to survive several months on their own reserves (Hölldobler and Wilson 1990). Likelihood of survival is high, nevertheless will decrease with increasing travel duration. Multiplication of a colony during spread within the EU cannot be ruled out, but is rather unlikely.
2.6a. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Horticultural plants and products and soils/substrates are usually not treated before translocation within the EU.
2.7a. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Fully developed nests are quite visible. In contrast, newly-founded nests with few queen(s) and workers can easily travel undetected in soil or other horticultural products.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	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.
2.9a. Estimate the overall potential for rate of spread within the Union based on this pathway (when possible provide quantitative data)?	very slowly slowly **moderately** rapidly very rapidly	low medium **high**	We consider this pathway as the most likely pathway of spread of *S. geminata* within Europe. A similar conclusion has been made for New Zealand (Harris 2005). The rate of spread will depend on the internal volume of trade within Europe. 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).
Pathway name:	b) Transport-Stowaway (Container/bulk, including road transport, sea freight, airfreight, train, etc.)		
2.3b. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	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.
2.4b. 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?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	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.
2.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Ant queens that independently found new colonies are able to survive several months on their own reserves (Hölldobler and Wilson 1990). Their likelihood of 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.
2.6b. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Most potential commodities that can carry ants or nests are not managed.

2.7b. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	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.
2.8b. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	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.
2.9b. Estimate the overall potential for rate of spread within the Union based on this pathway (when possible provide quantitative data)?	very slowly slowly **moderately** rapidly very rapidly	low medium **high**	Given the high numbers and types of commodities and items that can be associated with *S. geminata*, 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. 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).
Pathway name:	c) Unaided (Natural dispersal)		
2.3c. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	
2.4c. 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?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Spread by nuptial flights occur only during the warmest months of the year, and will likely be restricted to few weeks in the risk assessment area; it will include small numbers of alates, while budding usually includes a larger number of queens and workers. Queens will abort their mating flights in the presence of wind, which may indicate that their flights are

			focused on local rather than long distance dispersal (Bhatkar 1990). The likelihood of reinvasion after eradication is identical to the likelihood of introduction in the first place.
2.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Rates of survival of individual mated queens are relatively low after the nuptial flight (Hölldobler and Wilson 1990). However, this low life expectancy is compensated by the production of tens of females per nest. Dispersion by budding increases queen survival, however it remains to be observed in *S. geminata* polygynous colonies.
2.6c. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	There are no management practices currently in place.
2.7c. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely **moderately likely** likely very likely	low medium **high**	Low ant densities (e.g. single queens, 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 *S. geminata* has a painful sting, and is highly likely to be found in close association with urban areas and people should aid early detection of its presence, even if its initial establishment go unnoticed.
2.8c. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Queen ants can fly up to 2 km, and will likely find suitable habitats (e.g. sunny open habitat)

2.9c. Estimate the overall potential for rate of spread within the Union based on this pathway (when possible provide quantitative data)?	very slowly slowly **moderately** rapidly very rapidly	low medium **high**	*Solenopsis geminata* will spread unaided to all suitable habitats within its suitable climatic range. Alate females (queens) can fly up to 2 km during nuptial flights in monogynous colonies. This rate of spread decreases in polygynous colonies that reproduce by budding (below 300m per year, Hölldobler & Wilson 1990). For polygyne *S. invicta*, the invasion front moved 10.40 m/yr in central Texas via budding (Porter 1988). There are a number of intrinsic and extrinsic factors that influence spread including availability of disturbed habitats and morphology of the queens (Tschinkel 2006; King and Tschinkel 2008).
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	very easy easy with some difficulty difficult **very difficult**	low medium **high**	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 *S. geminata* become established in a European region, quarantine measures could be put in place to restrict the risk of long-distance spread, e.g. through nursery stock, as in USA for *S. invicta*.
2.11. Estimate the overall potential for rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	very slowly slowly **moderately** rapidly very rapidly	low medium **high**	Based on observations in introduced areas at its bioclimatic limits (e.g. US) where *S. geminata* has been replaced by *S. invicta* and 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. 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 depend on the internal volume of trade within Europe.

2.12. Estimate the overall potential for rate of spread in relevant biogeographical regions in foreseeable climate change conditions	very slowly slowly **moderately** rapidly very rapidly	low medium **high**	Climate change will not significantly increase the potential or speed of spread directly, as it is not expected to significantly widen the distribution range (98% of overlap between species' current and future potential distributions) (Bertelsmeier et al. 2015). However, it may facilitate population growth with subsequently increasing potential for spread.

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is 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?	minimal minor **moderate** major massive	low **medium** high	*Solenopsis geminata* is one of the most widespread invasive ant species but it is not considered as one of the worst. Indeed, the environmental impacts of *S. geminata* seem to be less pronounced than those of other invasive ants (Holway et al. 2002). Environmental impacts caused by the ant in the invaded ranged excluding the European Union are multiple: -<u>Impact on fauna</u>: In disturbed ecosystems at low latitudes in the New World (and other areas to which they have been introduced), *Solenopsis geminata* is often at the top end of dominance hierarchies (Morrison 1996). However, in Central America, *S. geminata* is a pioneer species colonising quickly after disturbance and initially dominant, but it is gradually replaced by other species after about 3 weeks (Perfecto 1991). In New Caledonia,

			S. geminata co-occurs with several other native and introduced species in open habitats (Blight et al. in prep). In La Réunion island, no impact on the fauna has been attributed to *S. geminata* (Jacquot et al. 2017). Foraging ants also prey on vertebrates. They have been reported to attack and consume young birds in their nest or those that have fallen from their nest (Plentovich et al. 2009); and sting young tortoises and land iguanas on the Galapagos (Williams and Whelan 1991). However, no studies that quantified impacts of *S. geminata* on vertebrate populations were found. The paucity of reports of effects of *S. geminata* compared to *S. invicta* suggests that attributes other than its stinging ability may explain the difference in the magnitude of their respective impacts. <u>-Impact on plants</u>: The impact on wild plants has been less studied than that on animals or cultivated plants. *Solenopsis geminata* interferes with seed dispersal of myrmecochorous plants by reducing dispersal distances, feeding on seeds, and leaving them exposed on the soil surface (Holway et al. 2002; Ness and Bronstein 2004). -<u>Alteration of ecosystem functions</u>: As with other invasive ant, *S. geminata* is attracted to plants by their carbohydrate-rich resources or by honeydew-producing herbivores. It has also been reported that *S. geminata* preys on Asian corn borer, *Ostrinia furnacalis* eggs and larvae, which might reduce pest infestation (Litsinger et al. 2007). It affects mutualistic interactions between plants and insects by

			reducing numbers of plant mutualists that protect the plant or disperse plant seeds (Ness and Bronstein 2004).
2.14. 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)?	minimal minor moderate major massive	low medium high	N/A. Because the species is not present in Europe, there is no current impact on biodiversity and related ecosystem services.
2.15. 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?	minimal minor **moderate** major massive	low **medium** high	It is likely that, if *S. geminata* establish and spread in the Mediterranean biogeographical region, the impact on native biodiversity, in particular on arthropods, and small vertebrates may be moderate to locally major and similar to that it is observed in presently invaded areas elsewhere.
2.16. 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?	minimal minor moderate major massive	low medium high	N/A. Because the species is not present in Europe, there is no current impact on the conservation value of native species or habitats.
2.17. 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?	minimal **minor** moderate major massive	low **medium** high	Although *S. geminata* can inhabit a wide range of open habitats, in invaded regions it particularly dominates highly disturbed habitats, such as newly deforested areas, road sides, agricultural areas including irrigated soils, gardens, etc. Therefore, many natural habitats of high conservation value may not be threatened by the ant. However, some open natural habitats in the Mediterranean biogeographical region may well be suitable.
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	minimal minor **moderate** major massive	low **medium** high	<u>Provisioning-Nutrition</u>: Foragers tend honeydew-producing homoptera, especially mealybugs, and including root feeding species. Homopteran tending may increase pest populations and reduce crop seed set and yields (Behera et al. 2001, cited in Harris 2005).

			Experimental removal of *S. geminata* from plots in an agroecosystem reduced aphid populations significantly (Risch and Carroll 1982). Regulating-Seed dispersal: *S. geminata* may interfere with seed dispersal of native ant species and directly predate, and therefore reduce the amount of seeds. However, it can, in some specific cases, contribute to disperse native plant species (Blight et al in prep.). Regulating-Pest and disease Control: *S. geminata* may interfere with beneficial insects that exert biocontrol activities in modified habitats. However, in several cases, *S. geminata* has been reported to provide benefits to crops by preying on pests (Way et al. 2002; Litsinger et al. 2007; Jacquot et al. 2017). Cultural-Physical use of landscapes: *Solenopsis geminata* is a social nuisance in infested areas. *S. geminata* colonies are common around urban areas and are considered urban pests in many countries (e.g., India (Lakshmikantha et al. 1996), USA (Smith 1965), and Hawaii (Reimer et al. 1990) cited in Harris 2005). In addition to stinging, foragers are attracted to electric fields (MacKay et al. 1992) and their chewing can cause damage to PVC coatings of electrical wiring potentially causing electrical shorts and resultant fires. They also build mounds in lawns, steal seeds from seedbeds, and enter buildings and feed on a range of household foods (Lee 2002, cited in Harris 2005).
2.19. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions	minimal minor moderate major	low medium high	N/A. Because the species is not present in Europe, there is no current impact on ecosystem services.

where the species has established in the risk assessment area (include any past impact in your response)?	massive		
2.20. 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?	minimal minor **moderate** major massive	**low** medium high	It is likely that, if *S. geminata* finds suitable habitats and climates for its development in the Mediterranean biogeographical region, the impact on ecosystem services may be moderate to locally major and similar to that observed in presently invaded areas. But its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability.
Economic impacts			
2.21. 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	minimal minor **moderate** major massive	low **medium** high	*S. geminata* is considered to be an economically important pest ant in some introduced areas however, data on the overall estimate of economic losses are unavailable. Losses in agricultural crops can be significant where this species is abundant. Foragers have been recorded feeding on the seeds and seedlings of sorghum, tomato, citrus, avocados, coffee, cocoa, corn, and tobacco (Risch and Carroll 1982; Lakshmikantha et al. 1996). These losses can be significant (e.g., 11% of potato and tomato crops had gnawed tubers and girdling of stems (Lakshmikantha et al. 1996)). Economic benefits can also be provided by this species; it has been documented to be a major predator of many other arthropod pests, may also be a valuable predator of weed seeds in some instances. It has for example reduced 98% of the population of the pest weevil *Sitophilus sp*. in corn crops (Risch and Carroll 1982) (see Q 2.18). <u>Health impacts</u>: *S. geminata* can sting people and may cause an allergic reaction that requires medical care and, sometimes, causes anaphylaxis. This ant has a

			painful sting that may cause injury to humans and domestic animals (Potiwat et al. 2018). However, the venom is chemically different to that of *S. invicta* (Cabreraa et al. 2004) and considered less potent (Taber 2000), and foragers behave less aggressively. This makes *S. geminata* less medically important. -<u>Impacts on infrastructure and equipment</u>: Ants and their mounds damage roads and electrical equipment. . Colonies move into buildings or vehicles seeking favourable nesting sites and as a result, domestic electrical equipment may be damaged such as computers, swimming pool pumps, cars or washing machines. *S. geminata* activities can result in the failure of many types of mechanical (such as hay harvesting machinery and sprinkler systems) and electrical equipment (Harris 2005).
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	minimal minor moderate major massive	low medium high	N/A. Because the species is not present in Europe, there is no current cost of damage.
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	minimal minor **moderate** major massive	**low** medium high	It is likely that, if *S. geminata* finds suitable habitats and climates for its development in the Mediterranean region, the economic cost may be moderate to locally major and similar to that observed in presently invaded areas. But its extent is very difficult to estimate considering the uncertainty related to habitat/climatic suitability.
2.24. 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)?	minimal minor moderate major massive	low medium high	N/A. Because the species is not present in Europe, there is no current cost of damage.

2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	minimal minor **moderate** major massive	**low** medium high	It is likely that, if *S. geminata* establish and spread in the Mediterranean and South 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.
Social and human health impacts			
2.26. 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).	minimal minor **moderate** major massive	low **medium** high	*Solenopsis geminata* is a social nuisance in infested areas. Colonies are common around urban areas and are considered an urban pest in many countries (e.g. India, USA, and Hawaii (Harris 2005)). Ants also enter buildings, destroying various domestic equipment. This ant has a painful sting that may cause injury to humans and domestic animals (Potiwat et al. 2018). The sting may produce an immediate, intense pain followed by red swelling. However, the venom is chemically different to that of *S. invicta* (Cabreraa et al. 2004) and considered less potent (Taber 2000), and foragers behave less aggressively, which makes *S. geminata* less medically important. *S. geminata* has been recently described as a vector of foodborne pathogens such as coliforms, *Bacillus spp*. or *Escherichia coli* (Simothy et al 2018). It may act as disease vectors and contaminate food, water and food-contact surfaces of kitchens resulting in foodborne illnesses.
2.27. 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.	minimal minor **moderate** major massive	**low** medium high	It is likely that, if *S. geminata* establish and spread in the Mediterranean region, the social impact, including health impact, may be locally moderate to major, and similar to that observed in presently invaded areas elsewhere.

Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	**NA** minimal minor moderate major massive	low medium high	*Solenopsis geminata* is not known for being used as food or feed, being a host or vector of other damaging organisms.
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	**NA** minimal minor moderate major massive	low medium high	No other impacts were found.
2.30. 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?	minimal minor **moderate** major massive	low **medium** high	There are no specific natural enemies of *Solenopsis* spp. in Europe. Thus, only generalist natural enemies of ants may affect the ant and these are highly unlikely to regulate (control) populations.

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ANNEXE

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
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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.

[10] Not to be confused with „no impact“.

Moderate	Measureable long-term damage to populations and ecosystem, but 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)

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.

<table>
<tr><th>Section</th><th>Division</th><th>Group</th><th>Examples (i.e. relevant CICES "classes")</th></tr>
<tr><td rowspan="6">Provisioning</td><td rowspan="6">Biomass</td><td>Cultivated terrestrial plants</td><td>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.</td></tr>
<tr><td>Cultivated aquatic plants</td><td>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.</td></tr>
<tr><td>Reared animals</td><td>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</td></tr>
<tr><td>Reared aquatic animals</td><td>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</td></tr>
<tr><td>Wild plants (terrestrial and aquatic)</td><td>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.)</td></tr>
<tr><td>Wild animals (terrestrial and aquatic)</td><td>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</td></tr>
</table>

			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[11]	**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.*

[11] 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.

	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** 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

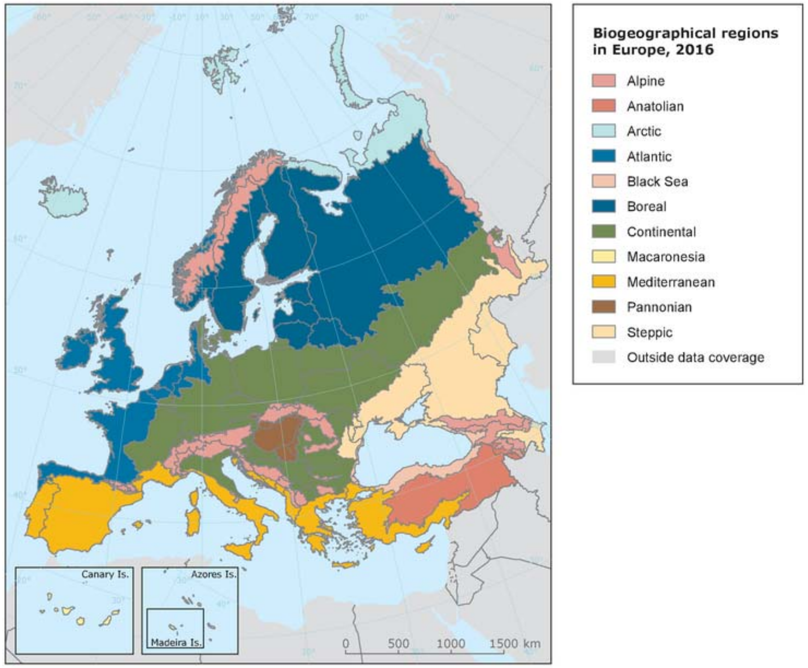
			Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific 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.*

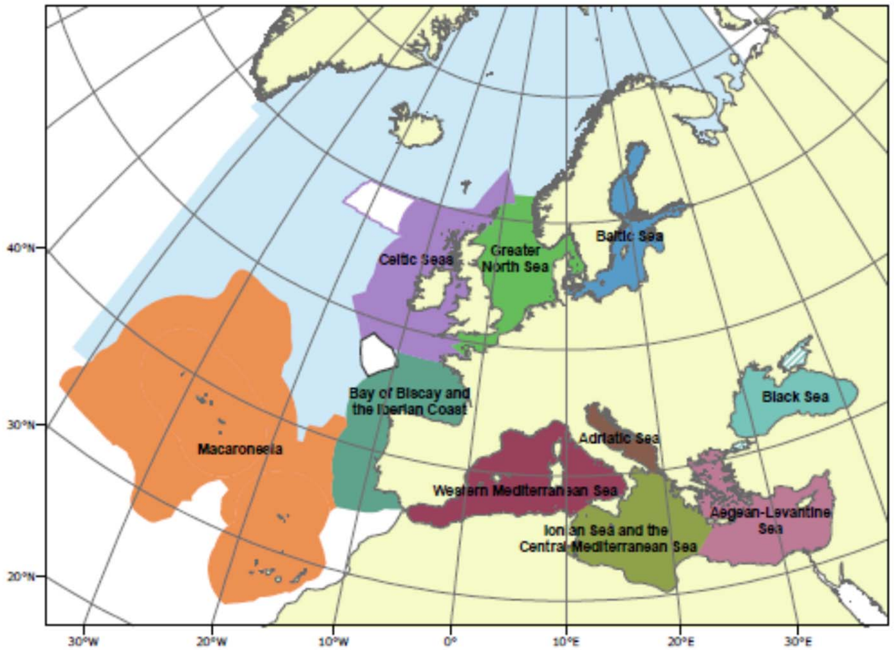
ANNEX V EU Biogeographic Regions and MSFD Subregions

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

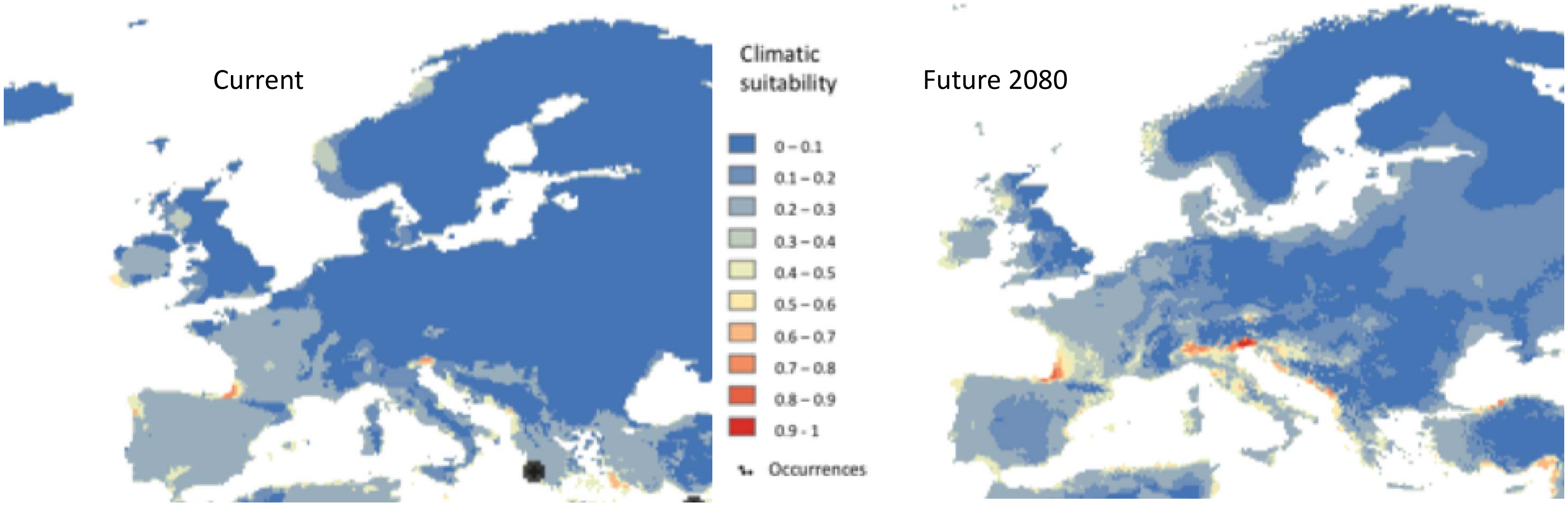
and

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





ANNEX VI Species distribution models under current and future (2080) climatic conditions (Bertelsmeier et al 2015).



Template for Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Solenopsis geminata***
Species (common name)	**The Tropical Fire Ant**
Author(s)	**Olivier Blight**
Date Completed	**10/18/2018**
Reviewer	**P. Robertson, R. Shaw**

Summary

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 USA and China 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.

There is probably no single method that will allow, alone, the control of *S. geminata* if this latter is introduced in Europe. However; currently the most effective control methods use chemical insecticides. Eradication of single nests in buildings, contained environments and containers is fairly straightforward and can be achieved at low cost. In areas where the climate is suitable for outdoor survival, efforts should be made to eradicate the nest(s) before queens escape into the wild. If *S. geminata* is already established and has begun to spread when first detected, management plans that consist in several applications of chemical insecticides per year over three to four consecutive years, followed by at least two years of intensive surveillance have to be adopted. Countries should have lists of chemical and biochemical insecticides authorised against invasive ants (as bait or contact) ready for use in case an invasion is detected. Chemical control is best when integrated into an IPM system that will reduce the volume needed. Research on biological control should be developed and may constitute a good complement to chemical control.

The management of invasive ants and particularly of S*. geminata* suffers from a lack of operational management experience. This lack of experience with this species increases the uncertainty when defining the most cost-effective measures.

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	**Inspection of imported goods and containers and destruction of nests and ants found at inspection.** 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 impossible, the selection of goods to inspect should consider their nature but also 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 *S. geminata* is present. However, invasive ants do not only arrive from the area of origin of	To reduce the chances of establishment of exotic ants in Europe, it is necessary to prevent their accidental entry. At the global scale, the number of introduced species in temperate regions is considered to be three and half times higher than the number so far detected (Miravete et al 2014), which highlights the need to set up a common detection method at ports and airports at a European scale. Quarantine inspections and treatments methods used in the USA and China could be adopted in Europe. Similar guidelines as those from USDA (2010, 2015) should be developed for invasive ants in general. 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 ants intercepted at inspections. However, 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 is needed, combined with a more risk-based approach to better target high risk items. To increase the efficiency of prevention efforts, a careful inspection of goods at port-of-exit should be combined with an active prevention mechanism at ports-of-entry to prevent contamination. New Zealand is likely the most proactive jurisdiction preventing exotic species	Medium

	the species but also via other localities (Bertelsmeier et al 2018) (see Wetterer 2010 for the species introduced range). In addition, in some cases, the species travels in goods and containers that transit via non-infested regions (Ma et al. (2010) in Wang et al. (2013)). To increase efficiency in methods to achieve prevention, a careful inspection of goods at port-of-exit should be associated to an active prevention at ports-of-entry. *S. geminata* may not be easily recognised by inspectors but all ant species, in particular queens and nests, should be destroyed immediately. USDA (2010) and USDA (2015) provide guidelines on how to treat infested commodities at ports of entry. This can involve immersion or dip treatment, drench treatment, topical treatment and incorporation of granular insecticides into potting media. Besides visual inspection, baiting is a cost-effective method of fire ant detection in China, but different techniques are required for specific goods (Hwang et al. 2009; Wang et al. 2013). In addition, the use of sniffing dogs is possible and might be a labour- and cost-efficient method of fire ant detection (e.g. Lin et al. 2011).	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; inspection actions have been reduced by 850 hours per annum, 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 *S. geminata*.	

Methods to achieve eradication	**Mechanical control.** When single nests are found in a confined container, e.g. a flower pot, destruction can be done manually through heat or freezing treatments (USDA 2010, 2015). However, to eradicate outdoor, established populations, chemical insecticides are the only effective method for use.	Mechanical control methods are clearly effective when dealing with small number of animals or individual nests, but are likely to be of limited use when dealing with more widespread populations in more open environments. No data are available on the costs and effectiveness of non-chemical eradication methods.	High
	Chemical control with insecticides. Eradication of single nests and, in particular, multiple nests, is best achieved using insecticides. Methods to kill single nests in containers such as potted plants, grass sod, baled hay, etc. are described in USDA (2015). They include immersion or dip treatment, drench treatment, topical treatment, and incorporation of granular insecticides into potting media. Single nests in buildings can also be destroyed using insecticide baits such as those commonly used to combat ants in buildings (Noordijk 2010). Eradication of established population outdoors is more problematic, especially when high numbers of nests are involved. The use of broadcast granular bait-formulated products is recommended. Eradication of *S. geminata* was possible across 3ha in northern Australia for at least two years mainly because small colonies were readily located within a	Eradication of single nests in buildings, contained environments and containers is rather straightforward and can be achieved at low cost. In areas where the climate is suitable for outdoor survival, efforts should be made to eradicate the nest(s) before queens escape in the wild. A key component to the success of the eradication program conducted in Australia was that it was supported and funded to its conclusion (approximatively AU$60 000 for 3ha treated) with good cooperation between the numerous stakeholders within the area targeted (Hoffmann and O'Connor 2004). This is the only successful documented attempt to eradicate *S. geminata*. There is much more information available on the management of *Solenopsis invicta.* The results of outdoor eradication programmes targeting *S. invicta* have been variable (see Kean et al. 2017 who describe 12 eradication programmes). The ant has been eradicated from various areas, including from climatically suitable ones (e.g. New Zealand and parts of Australia and Taiwan), but many eradication attempts failed (in various areas in USA, Australia and China). The eradication plan that has been put in place in Australia in early 2000s has cost so far about AUS$300 million for treating an area of nearly 70,000 ha. It has achieved the eradication of at least two incursions but others have not yet been eradicated, although at least one of them is now under containment (Invasive Species Council 2015). The primary reasons for the failure of eradication attempts in USA include: (i) the inability to attain absolute (100%) control using available	High

	relatively restricted disturbed habitat (Hoffmann and O'Connor 2004). The species was treated with toxic granular baits delivered by hand. A list of eradication programmes carried out against *S. invicta* outdoors is provided in the GERDA database (Kean et al. 2017), which also lists techniques and products used for the eradication and references. Although examples of success and failures in attempts to eradicate *S. invicta* are noteworthy for *S. geminata* eradication, their distinctive diet implies adaptation in the methods employed. *S. geminata* includes more seeds in its diet which can affect attractiveness to the chemical delivery form commonly used for *S. invicta*.	products; (ii) the large area of infestation; (iii) high cost of treatment; (iv) inability to uniformly treat an entire area of infestation (Drees et al. 2006); and (v) the ability of fire ants to rapidly spread even before eradication efforts are put in place (Drees et al 2013). In Taiwan, an infestation of 13 Ha with a total of 1,578 mounds was successfully eradicated within one year. However, eradication programmes that were most successful were those involving one or a small number of nests, such as the two successes achieved in New Zealand (Christian 2009). The eradication of *S. invicta* in early 2000s in Auckland covered less than 1 Ha but cost NZ$1.4 million. In Australia, an eradication programme of *S. invicta* was evaluated at AU$200 million (Hoffmann et al 2010). This programme had noteworthy success, and highlighted valuable lessons. For example, the programme revealed clear differences in the efficacy of bait application; aerial application has proven to be the most efficient strategy followed by hand and land vehicles application methods. It is of utmost importance to start eradication programmes as soon as possible. Therefore, it is essential to develop contingency plans against this and other invasive ants at the European scale to be ready when ant establishments are notified. These plans should include considerations on social and environmental issues related to the use of chemical controls as well as lists of products licensed for ant control indoors and outdoors.	
Methods to achieve management	**Chemical control.** Chemical control will target not only the worker but also, and importantly, the queen, to kill nests. Options include broadcast granular bait-formulated products, treatment of individual ant colonies in mounds and surface or barrier treatments using contact insecticides (Drees and Gold 2003; Drees et al. 2013; CABI 2017). Common insecticides that can be used	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. Data on the management costs of *S. invicta* using insecticides in USA are available (Barr et al. 2005). Conventional bait insecticides cost approximately US$10 per 0.4 ha for broadcast application, and with the	High

	for fire ant control in USA are provided by Drees et al. (2013) and Greenberg and Kabashima (2013). Different insecticides will be used for the different options. Drees et al. (2013) also discuss the limitations of chemical treatments and their integration into an IPM system. Wang et al. (2013) reviews research in China on chemical and other control methods *against S. invicta*. Of interest is the effective use of bioinsecticides (e.g. spinosad and plant extracts) and the good results obtained using the two-steps approach, i.e. first a bait is broadcasted over large areas and, then, remnants of ant mounds are treated individually with contact insecticides.	cost of application, total treatment costs are 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).	
	Cultural and sanitary methods. Cultural management methods in cattle production have been developed to limit damage of *S. invicta* to livestock. For example, the use of disc-type cutters, the quick removal of hay bales from the field and the scheduling of cow fertility programmes to avoid calving during hot, dry summer months (Drees et al. 2013; CABI 2017). Other approaches can be used in different environments. In particular, it has been shown that frequent disturbance of mounds causes colonies to move to less disturbed areas (Drees et al. 2013). More generally, hindering favourable habitats may be considered. Disturbed	These and other cultural and sanitary methods could be considered for use in Europe. There is no information on the cost-effectiveness of these methods.	Medium

	areas should be allowed to regenerate after which the vegetation succession will make the site less suitable for *S. invicta* (Noordijk 2010).		
	Biological control. The biological control of *S. geminata* is only at its beginning, no species has been specifically tested. Current research is identifying parasitoids and pathogens in its native range (Plowes et al. 2009). In contrast, many natural enemies of *S. invicta* have been identified. They occur in its native range and are believed to keep populations at lower densities than in the invaded regions. Many of them have been studied for their potential as classical biological control agents. Several parasitic flies of the genus *Pseudacteon* (Diptera: Phoridae) have been introduced and have established and spread in the USA since 1997 (Graham et al., 2003; Williams et al. 2003; Morrison 2012). However, it seems that the different species of *Pseudacteon* tested are more attracted by *S. invicta* than by *S. geminata,* which may decrease their potential to control the Tropical Fire Ant (Estrada et al. 2006). In addition, microsporidia and viruses have been studied in several *Solenopsis* species including *S. geminata*, and at	*Pseudacteon* spp. and the pathogens could possibly be considered for introduction to Europe since they are specific to one or a few exotic *Solenopsis* spp and should therefore have limited side effects on the environment, with maybe the exception of native *Solenopsis spp* (Folgarait et al. 2002; Oi and Valles 2012). However, so far, the effect of *Pseudacteon* spp. on *S. geminata* population densities has not been demonstrated (Morrison 1999; Morrison et al. 2000), even in *S. inivcta* the main target of such biological control, possibly because average parasitism rate per colony is too low (Morrison and Porter 2005; Tschinkel 2006; Morrison 2012). Some data on the cost of releasing *Pseudacteon* spp in the USA are available in Drees et al. (2013) but they are not really applicable to Europe since they do not include the necessary significant pre-release investigations. The production cost of *Pseudacteon* is estimated at $1.00 per fly. Five thousand flies were released near Gainesville (Florida) in 1997. By fall of 2005, they spread to over 90 000 square kilometres (Drees et al. 2013). The cost of this release was estimated at $10 000, but considering the spread of the species, treatment cost estimate dropped down to at $0.0001/ha. Classical biological control, i.e. the introduction of exotic natural enemies for their permanent establishment and long-term control of a pest, is a very cost-effective method since no action is required after releases and establishment. However, the level of control that would be achieved through the release of the flies is very uncertain. In the Southern USA, it is thought that, collectively, these natural enemies may help reduce the frequency of insecticide applications required to maintain *S. invicta* control, but are not sufficiently effective to achieve control on their own (Oi et al. 2007; Drees et al 2013).	Medium

	least a microsporidia, *Kneallhazia solenopsae*, has been found established in North America (CABI 2017). A molecular analysis revealed that *Kneallhazia solenopsae* infects *S. geminata* but its impacts on colony survival are unknown (Ascune et al. 2010). The entomopathogenic fungus *Beauveria bassiana sensu lato* has also been applied against *S. invicta* in the field in established mounds (e.g. Bextine and Thorvilson 2002). Whereas the efficacy of this method has been demonstrated under laboratory conditions, few studies validated its use in the field. However, Bextine and Thorvilson (2002) conducted two field experiments, one at the mounds scale and another at a site scale (700m^2). In both experiments they successfully inactivated up to 80% of the mounds. Similarly, Kalfe et al. (2010) succeed in inactivating 70% (22 treated) of the mounds treated with *B. bassiana*. In both studies, the most efficient delivery form was the use of baits (e.g. fungal pellets coated with peanut oil) instead of a direct application of the fungus.	The different agents also affect the species in different ways which influences their possible effectiveness. *Pseudacteon* spp. parasitize a small percentage of workers but indirectly affect colonies by suppressing daytime foraging behaviours whereas disease organisms directly affect ants and colony health (Drees et al. 2013).	
	Integrated pest management. To keep population levels below those that cause economic, social, or ecological damage, the integration of chemical, cultural, biological and regulatory methods into an IPM system is needed	There is probably no single method that will allow, alone, the control of *S. geminata* if this latter is introduced in Europe. The long expertise gained in USA on the development of IPM programmes against *S. invicta*, reviewed and analysed in Drees and Gold (2003) and Drees et al. (2013) will undoubtedly help developing specific programmes for Europe. Their cost is impossible to assess in the present situation.	High

	(Hoffmann et al 2010). Drees et al. (2013) provide the latest information on IPM methods developed against *S. invicta* in the Southern USA. IPM design considerations include management goal(s), action level(s), ant form (monogyne or polygyne), presence of nontarget ant species, size of treatment area, seasonality, implementation cost, and environmental impact are also presented. Their conclusion is that "There is no single best IPM program for imported fire ants. Programs designed and implemented using IPM concepts will vary due to multiple factors including the presence and abundance of fire ants and other ant species, together with the level and seasonality of control desired, established natural enemies in the management area, availability of registered insecticide products for the use sites involved, environmental concerns, and cost of application(s) that include time and labour. Optimally, elegant IPM programs would be target specific, threshold driven, environmentally friendly and cost-effective. With eradication unlikely to succeed in areas larger than isolated spot infestations, containment and suppression become the overriding goals. However, within such larger landscapes, maximum control is attainable using well-designed treatment programs that, where		

	justified, periodically use selected chemical methods."		

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2[1]

Name of organism: *Cydalima perspectalis* (Walker, 1859)

Author(s) 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: Marianne Kettunen, Institute for European Environmental Policy, London, United Kingdom
Peer review 2: Dr Gábor Vétek, Department of Entomology, Szent István University, Budapest, Hungary
Peer review 3: Dr Archie Murchie, Agrifood and Biosciences Institute, Northern Ireland, UK

This risk assessment has been peer-reviewed by three independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study.

Date of completion: 25 October 2018

Photo credit: Tim Haye

[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

Contents

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**[2]	**COMMENT**
Summarise Entry[3]	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The organism is already present in the risk assessment area and in most EU countries.
Summarise Establishment[4]	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The organism is already established in the risk assessment area and most EU countries. Of particular importance is its probable absence in Mallorca where the rare *Buxus balearica* occurs. It is not clear whether the moths has reached the B. balearica stands in Andalucía and Sardinia.
Summarise Spread[5]	very slowly slowly moderately **rapidly** very rapidly	low medium **high**	The spread in Europe as contaminant of host plants and by natural dispersal has been very fast. In 12 years it invaded most of the suitable areas in Europe. It probably still has to reach some native stands of *Buxus* spp. in Southern France and Spain.
Summarise Impact[6]	minimal minor moderate major **massive**	low medium **high**	While the economic impact of the invasion of *C. perspectalis* in Europe can be considered as currently minor, the ecological impact on biodiversity and, potentially, various ecosystem services is major to massive. Natural stands of *Buxus sempervirens* are quickly disappearing, potentially leading to the local extinction of a high number of species closely linked to

[2] In a scale of low / medium / high, see Annex III

[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly

[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

			the plant.
Conclusion of the risk assessment[7]	low moderate **high**	low medium **high**	*Cydalima perspectalis* is already present in most of its potential range, which covers most natural stands of native *Buxus* spp. in Europe. If no area-wide management method is implemented to lower populations in natural stands, e.g. through the introduction of a specific natural enemy from Asia, or if no resilience of *Buxus* stands are observed in the next few years, the risk is high that whole ecosystems will disappear, including many species that live exclusively in these ecosystems.

[7] In a scale of low / moderate / high

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established (future)	Invasive (currently)*
Austria		Yes	Yes	
Belgium		Yes	Yes	Yes
Bulgaria		Yes	Yes	
Croatia		Yes	Yes	
Cyprus			Yes	
Czech Republic		Yes	Yes	
Denmark		Yes	Yes	
Estonia			Yes	
Finland			Yes	
France		Yes	Yes	Yes
Germany		Yes	Yes	Yes
Greece		Yes	Yes	
Hungary		Yes	Yes	
Ireland			Yes	
Italy		Yes	Yes	Yes
Latvia			Yes	
Lithuania			Yes	
Luxembourg		Yes	Yes	
Malta			Yes	
Netherlands		Yes	Yes	
Poland		Yes	Yes	
Portugal		Yes	Yes	
Romania		Yes	Yes	

Slovakia		Yes	Yes	
Slovenia		Yes	Yes	
Spain		Yes	Yes	Yes
Sweden		Yes	Yes	
United Kingdom		Yes	Yes	

*Countries where damage on wild box stands has been observed

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)*
Alpine	Yes	Yes	Yes	
Atlantic	Yes	Yes	Yes	
Black Sea	Yes	Yes	Yes	
Boreal			Yes	
Continental	Yes	Yes	Yes	Yes
Mediterranean	Yes	Yes	Yes	Yes
Pannonian	Yes	Yes	Yes	
Steppic	?	?	Yes	

*Regions where damage on wild box stands has been observed

ANNEXES

ANNEX I Map of predicted distribution and relative abundance (Ecoclimatic Index) of *Cydalima perspectalis* in Europe.

ANNEX II Map of occurrence of natural stands of *Buxus sempervirens* and *B. balearica* in Europe.

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Scientific name: *Cydalima perspectalis* (Walker, 1859) Class: Insecta Order: Lepidoptera Family: Crambidae Genus: *Cydalima* Lederer, 1863 Synonyms: The species was previously placed in various genera, most commonly *Diaphania* and *Glyphodes*. Mally and Nuss (2010) most recently placed it in the genus *Cydalima*. *Diaphania perspectalis* (Walker, 1859) *Glyphodes perspectalis* (Walker, 1859) *Palpita perspectalis* (Walker, 1859) Common names: Box tree moth, box tree caterpillar, pyrale du buis (F), Buchsbaumzünsler (D) No sub-species, varieties or breeds are known.
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 wild, in confinement or associated with a pathway of introduction]	According to Mally and Nuss (2010), there is no risk to confuse this species with any other Crambidae. Furthermore, in the EU there is no similarly looking caterpillar on *Buxus* spp.
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	Yes, FERA did a rapid risk assessment for UK in 2010 (FERA 2010). The conclusion was that there was no need for a full pest risk analysis since it was already present in the UK and already too widespread in Europe. They also added that, without interventions, impacts are likely to be seen in *Buxus* plants across Europe. However, this RA did not specifically consider the risk for wild box trees in Southern Europe,

	which were not yet affected at that time.
A4. Where is the organism native?	*Cydalima perspectalis* is supposedly native to India, China, Korea, Japan and the Russian Far East (Mally and Nuss 2010). According to Nacambo et al. (2014), the presence of the moth in India refers to an old reference only (Hampson 1896) and its presence is uncertain. It could be also non-native in regions where *Buxus* spp. do not occur naturally, such as Northern China and Russian Far East. Most records in the literature refer to ornamental *Buxus* spp. in urban areas. Its confirmed distribution in Asia covers a wide variety of climates from the humid continental climate of North-Eastern China and Russian Far East to the humid subtropical climate of Southern China and southern Japan.
A5. What is the global non-native distribution of the organism outside the risk assessment area?	Outside the risk assessment area, it is distributed in most European non-EU countries (see below) as well as Turkey (Hizal 2012), Georgia (Matsiakh 2014), Iran (Zamani et al. 2017) and Western Russia (Tuniyev 2016). It has been notified in Pakistan by Sial et al. (2017) but it cannot be ruled out that it is native in this country. *Cydalima perspectalis* is present in non-EU countries in Central Europe such as Switzerland and Liechtenstein as well as in Southern Europe such as Bosnia-Herzegovina, Albania, Macedonia, Montenegro and Serbia (Raineri et al. 2017; CABI 2018; M. Kenis unpublished data).
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?	*Cydalima perspectalis* is recorded and established in the following terrestrial biogeographic regions in the risk assessment area: Atlantic, Black Sea, Continental, Mediterranean and Pannonian (CABI 2018). It is also present at lower altitudes in the Alpine region; it is likely present in the Steppic region although not clearly reported; it is still probably absent from the Boreal region.
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?	*Cydalima perspectalis* is already present in most of its potential range in the risk assessment area. With climate change, it should be able to establish at higher altitudes in the Alpine region and in warmer areas of the Boreal region, although no model has been used yet to predict its distribution in climate change scenarios. Of particular importance is its apparent absence in most Mediterranean Islands, including the Balearic Islands (Mallorca) where the rare *Buxus balearica* occurs. For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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.	Recorded in the following Member States: Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, Luxembourg (Hellers and Christian 2016), Netherlands, Poland (Kudła and Dawidowicz 2016), Portugal (Corley et al. 2018), Romania, Slovakia, Slovenia, Spain, Sweden (Bengtsson 2017), United Kingdom. When no reference is given, see CABI (2018).

	The moth was first reported from Germany and the Netherlands in 2007, from observations dating from 2006 (Krüger, 2008; Straten and Muus, 2010). Then, in less than 10 years, it spread rapidly to the other countries mentioned above.
A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	No records were yet found from Cyprus, Estonia, Finland, Ireland (but a moth has been caught in Northern Ireland), Latvia, Lithuania and Malta. The moth can probably establish in all these member states under current climatic conditions, except for Finland, where the distribution is likely to be restricted to the warmest regions and more likely under foreseeable climate change. In several countries listed under A8, the moth is not yet known from the whole territory. In Sweden and UK, it is likely that the northern regions are not yet suitable for its establishment. In others (e.g. Spain and Portugal), it is only a matter of time before the whole country will be invaded.
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?	Yes. In the Caucasus, most particularly Georgia, it severely affects native stands of *Buxus sempervirens* (Tuniyev 2016; Matsiakh et al. 2018; Mitchell et al. 2018).
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	*Cydalima perspectalis* is damaging ornamental *Buxus* spp. in most areas where it has become established. However, in Northern Europe, where it cannot complete two generations, damage is much less severe (M. Kenis, unpublished data). It is invasive in the following biogeographic regions in the EU: Atlantic, Black Sea, Continental, Mediterranean and Pannonian (CABI 2018). Its only host plants, *Buxus* spp. occur naturally mainly in the Mediterranean and Continental regions of the EU (Di Domenico et al. 2012; Kenis et al. 2013). It is therefore in these regions that the species is likely to be most invasive.
A12. In which EU member states has the species shown signs of invasiveness?	Signs of invasiveness have occurred in most countries where it has become established, as listed in A8. Di Domenico et al. (2012) (see Annex II) and Kenis et al. (2013) provide maps of the occurrence of *Buxus sempervirens* and *B. balearica*, its only two potential wild hosts in Europe. EU countries where there have been notifications of *C. perspectalis* damaging wild stands of *Buxus sempervirens* include Belgium, France, Germany, Italy, and Spain (John and Schumacher 2013; Kenis et al. 2013; Raineri et al. 2017; Mitchell et al. 2018). The rare stands of *B. balearica* in Southern Spain and Mallorca have not yet been found infested. In Sardinia, the moth has been observed on cultivated *Buxus* spp. but it is not known whether it has reached the rare B. *balearica* stands (Prof Ignazio Floris, personal communication).
A13. Describe any known socio-economic benefits of the organism.	There is no known socio-economic benefit for this 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.”
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future	none very few **few** moderate number	low medium **high**	

[8] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf
[9] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

pathways respond N/A and move to the Establishment section)	many very many		
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	Contaminant on plants, Unaided		Entry pathways in the risk assessment area mainly consist in: a) Contaminant on plant: Import of live *Buxus* plants or plant parts into the EU b) Unaided: Adult flight from neighbouring countries.
Pathway name:	Contaminant on plants (Import of live *Buxus* plants or plant parts into the EU).		
1.3a. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional **unintentional**	low medium **high**	
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	*Cydalima perspectalis* has already been introduced into the EU (from Asia) and into other parts of the world (e.g. into Russia from Italy) via this pathway. However, while millions of box tree plants were imported from Asia up to 2010 (EPPO 2012), the volume of trade has most probably largely decreased nowadays because of the low amount of *Buxus* spp. sold in Europe after the introduction of *C. perspectalis* and another invasive species, the fungus *Calonectria pseudonaviculata.* Furthermore, traded box plants are now usually all treated with systemic insecticides. Thus, the frequency of entries (propagule pressure) and the likelihood of large numbers of individuals are lower than they used to be, albeit difficult to quantify.

1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The insect is very resistant to different climatic conditions. Overwintering small larvae are the most likely transported stage and can survive several months in diapause or quiescence (Nacambo et al. 2014).
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	Box trees are supposed to be free of pests and treated against the moth and other insects with insecticides. It was already so when the species was introduced accidentally into Europe and other regions. However, management practices in Europe have increased since the outbreak of *C. perspectalis*, i.e. plants are usually treated with systemic insecticides before being sold.
1.7a. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Eggs and young larvae are difficult to detect on plants.
1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Since *C. perspectalis* travels on its plant, it will arrive in an appropriate situation for establishment.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Emerging adults from imported plants will be able to find other trees since box trees are frequently planted as ornamentals. However, natural *Buxus* spp. are not that common in the wild and usually far from ornamental *Buxus* plants. Thus, it may take some years before the moth reaches natural stands.
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	The chance of new introductions from Asia is lower than in the past because the volume of traded *Buxus* trees has decreased recently and management practices have increased, including in the risk assessment area.

Pathway name:	Unaided (Natural dispersal across borders of invasive alien species that have been introduced through pathways 1 to 5)		
1.3b. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional **unintentional**	low medium **high**	
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Adults are good flyers and, when swarming in large numbers during outbreaks, they can spread several kilometres by themselves (Leuthardt et al. 2010).
1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	*Buxus* spp. are commonly planted in parks and gardens and, thus, adults that would enter not yet colonized areas within the risk assessment area through natural dispersal would likely find trees for survival.
1.6b. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely likely very likely	low medium high	N/a
1.7. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	By nature, natural flights are largely undetected. However, adults are highly attracted to light sources as well as well as to pheromones, which can be used to monitor entries in new areas.
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely	low medium	Adults entering through natural flight will enter the risk assessment area during an appropriate season.

	moderately likely likely **very likely**	**high**	
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	*Buxus* spp. are commonly planted in parks and gardens and, thus, adults that would enter not yet colonized areas within the risk assessment area through natural dispersal would likely find trees for ovipositing. However, natural *Buxus* spp. are not that common in the wild and usually far from ornamental *Buxus* plants and, thus, it may take some time before the moth reaches natural stands.
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	There are only few areas in the risk assessment area where the moth is not yet present and where the climate is suitable.
End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The likelihood of entry to not yet colonized areas in the risk assessment area by natural dispersal from neighbouring countries is very high.
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The areas not yet invaded in the risk assessment area because of climatic unsuitability (the largest parts of Fennoscandia) are more likely to be invaded in the future under foreseeable climate change.

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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The species is already established in most EU countries. Nacambo et al. (2014) provided a CLIMEX model for the moth and a risk map in Europe. Some areas that are climatically suitable are not yet fully invaded, in particular areas in Spain, Portugal and Southern Italy (Annex 1).
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The species is already established in most EU countries.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	very isolated isolated moderately widespread **widespread** ubiquitous	low medium **high**	*Cydalima perspectalis* is restricted to *Buxus* spp. in Europe (Leuthardt and Baur 2013; Matošević et al. 2017). Ornamental *Buxus* plants are widely planted in the EU. Natural *B. sempervirens* populations are more scattered and abundant only in Southern France and Northern Spain (Di Domenico et al. 2012; Annex 2). In the EU, *B. balearica* is a rare species only present in the Balearic islands (Mallorca), Andalusia and at one site in Sardinia.
1.16. 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?	very unlikely unlikely moderately likely likely	low medium **high**	In Europe, the organism is restricted to its host plant on which it feeds. Eggs and Larvae are transported together with the plants and establishment is therefore very likely.

	very likely		
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	There is no other significant defoliator on *Buxus* spp. in Europe. However in some regions, *Buxus* spp. are also severely affected by box blight (caused by *Calonectria pseudonaviculata* (=*Cylindrocladium buxicola)* and *C. henricotiae*) two invasive fungi, and other indigenous fungi, which may also severely damage *Buxus* plants, including in wild stands (Lehtijärvi et al. 2014; Gehesquière et al. 2016).
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Parasitism by indigenous parasitoids is very low (Wan et al. 2014). It is commonly attacked by generalist predators such as wasps and birds (M. Kenis, unpublished data) but so far it has not affected populations.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Management practices are in place for ornamental trees, e.g. using pesticides or biological control products, with local success, but without preventing establishment. There is no management practices already in place for natural *Buxus* stands.
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	**very unlikely** unlikely moderately likely likely very likely	low medium **high**	Cutting/uprooting and careless disposal of trees or parts of trees favours the spread of the species.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Most larvae are killed by pesticides or biological control products such as *Bacillus thuringiensis*. There are yet no indications of resistance or avoidance behaviour. However, the high reproduction rate and the already wide distribution in the risk assessment area make it very likely to survive local or regional eradication attempts.
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	very unlikely unlikely moderately likely	low medium **high**	Its biological characteristics have facilitated its establishment: -High fecundity, i.e. 199-488 eggs/female

	likely **very likely**		depending on the generation (Cheng 2005, in Wan et al. 2014) -Two generations per year in Central Europe, at least three in southern Europe (Nacambo et al. 2014) -Good flight capacities (several km per year), allowing the adult to find suitable trees for oviposition (Leuthardt et al. 2010)
1.23. How likely is the adaptability of the organism to facilitate its establishment?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The species is already established in most EU countries.
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The species is already established in most EU countries. The high and geographically structured genetic diversity observed in Europe suggests multiple introductions events (Bras et al. 2016).
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The species is already established in most EU countries.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	very unlikely unlikely moderately likely likely very likely	low medium high	N/a. The species is already established in most EU countries.
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	*Cydalima perspectalis* is established in the following terrestrial biogeographic regions in the EU: Atlantic, Black Sea, Continental, Mediterranean and Pannonian (CABI 2018). It is also present at lower altitudes in the Alpine region; it is likely present in the Steppic region

			although not clearly reported as established; it is still probably absent from the Boreal region. Only some areas in Southern Europe (Spain, Portugal, Greece, Southern Italy, Malta and Cyprus) are still to be invaded (Nacambo et al. 2014; Annex 1).
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	In foreseeable climate change conditions, the warmest areas of the Boreal Region may be invaded, as well as cool regions from the Alpine, Atlantic and Continental regions that are presently too cold for the moth to survive or to complete a generation. More important, with temperature increases, the moth will develop two generations per year in areas where it cannot presently complete two generations. This will most certainly cause higher damage in Northern Europe and at higher elevations (Nacambo et al. 2014).

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	minimal minor moderate **major** massive	low medium **high**	Adult moths are good flyers and are able to spread up to 7 km per year (Leuthardt et al. 2010).
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	minimal minor moderate **major** massive	low medium **high**	The moth has spread, and is still spreading in Europe via the plant trade. The trade of *Buxus* spp. is not regulated within the EU. There is no data on the trade of *Buxus* spp. in the EU. Individual persons and gardeners also transport *Buxus* spp. at shorter distances, including for the disposal of cut infested trees.
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	Contaminant on plants, Unaided		Entry pathways in the EU mainly consist in : a) Unaided: adult flight b) Contaminant: movements of live *Buxus* plants or plant parts in the EU.
Pathway name:	Unaided (Natural dispersal by adult flight)		

2.3. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	
2.4. 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?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Adult moths are good flyers, able to spread several km per year (Leuthardt et al. 2010) and due to its abundance it is very likely that sufficient numbers of individuals spread to originate viable populations.
2.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	*Buxus* spp. are commonly planted in parks and gardens and, thus, adults that would enter not yet colonized areas within the risk assessment area through natural dispersal would likely find trees for survival.
2.6. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely likely very likely	low medium high	N/a
2.7. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	Adult flights are nocturnal and largely undetected when invading a new area in low numbers. However, adults are highly attracted to light sources as well as well as to pheromones, which can be used to monitor the spread of the organism in new areas
2.8. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	*Buxus* spp. are commonly planted in parks and gardens in the EU and, thus, adults that would move through natural flight would very likely find trees for ovipositing.
2.9. Estimate the overall potential rate of spread within the Union based on this pathway? If possible, provide quantitative data.	very slowly slowly **moderately** rapidly very rapidly	low medium **high**	The species has already spread to large parts within the risk assessment area. Further spread by natural dispersal is likely. Adult moths are good flyers and are able to spread several up to 7 km per year (Leuthardt et al. 2010).

Pathway name:	Contaminant on plants (Movement of live *Buxus* plants or plant material)		
2.3. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	
2.4. 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?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Eggs, larvae and pupae can be very abundant on traded and non-traded *Buxus* plants or plant materials, although traded plants are now usually protected with insecticides.
2.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	When carried on their host plants, the insect can develop until adult emergence. The insect is very resistant to different climatic conditions. Overwintering small larvae are the most likely transported stage in winter and can survive several months in diapause or quiescence (Nacambo et al. 2014).
2.6. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Marketed box trees are usually, but not always, treated against the moth with systemic insecticides. Trees and branches carried by people can be infested.
2.7. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Eggs and young larvae are difficult to detect on plants.
2.8. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	When *C. perspectalis* travels on its live host plant, it will arrive in an appropriate situation for establishment. Emerging adults will be able to find other trees since box trees are frequently planted as ornamental plants. However, natural *Buxus* spp. are not that common in the wild and usually far from ornamental *Buxus* plants. Thus, it may take some time before the moth reaches natural stands.

2.9. Estimate the overall potential rate of spread within the Union based on this pathway? If possible, provide quantitative data.	very slowly slowly moderately **rapidly** very rapidly	low medium **high**	Live plants' movement is the most suitable long distance pathway in Europe and the most likely cause of the fast spread of the moth to most European countries in just about 10 years (Matošević et al. 2017). The movement of *Buxus* spp. plants is free within Europe. Traded plants are now commonly treated with insecticides but there are exceptions and private people are also carrying plants. Cut branches for celebrations and decoration and cut or uprooted trees brought to composting places can also contribute to the spread.
End of pathway assessment			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	very easy easy with some difficulty difficult **very difficult**	low medium **high**	Adult flights cannot be contained and the trade of *Buxus* spp. is not regulated within the EU.
2.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues). If possible, provide quantitative data.	very slowly slowly moderately **rapidly** very rapidly	low medium **high**	The few areas in the Mediterranean Region that are climatically suitable and not yet invaded will be reached within the next 5 years, with the possible exception of islands.
2.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions. If possible, provide quantitative data.	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	In foreseeable climate change conditions, the warmest areas of the Boreal Region may be invaded, as well as cool regions from the Alpine, Atlantic and Continental regions that are presently too cold for the moth to survive or to complete a generation. More important, with temperature increases, the moth will develop two generations per year in areas where it cannot presently complete two generations. This will most certainly cause higher damage in Northern Europe and at higher elevations (Nacambo et al. 2014).

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is 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?	minimal minor moderate major **massive**	low medium **high**	Total defoliation kills box trees very rapidly. In the Caucasus, the moth is eradicating *Buxus sempervirens* from large areas, an important component of natural forest ecosystems (Tuniyev 2016; Matsiakh et al. 2018; Mitchell et al. 2018). This probably has cascading effects on species that live exclusively or mainly in this ecosystem. Mitchell et al. (2018) found a total of 132 fungi, 12 chromista (algae), 98 invertebrate and 44 lichens using *Buxus* species in the Caucasus and Europe. Of these, 43 fungi, 3 chromista and 18 invertebrate species have only been recorded on *Buxus* species. This suggests that all these species are at risk if *Buxus* spp. were disappearing from the region. The impact has been scored "massive" in the context of box tree distribution and the intrinsically linked biodiversity, which will disappear in the absence of box trees.
2.14. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g.	minimal minor	low medium	In the risk assessment area, damage on native *Buxus sempervirens* stands that have been attacked since at

decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?	moderate major **massive**	**high**	least three years appear similar to those observed in the Caucasus, although the exact magnitude and long term effects on these stands still need to be confirmed. The natural stands that were first attacked in 2009 around Basel in Switzerland, Germany and France took about 8 years to decline by over 95% (John and Schumacher 2013; M. Kenis personal observation). However in this region the moth develops two generations per year while, in the Caucasus, where at least three generations per year are observed (as in Southern Europe), the decline was much faster (Tuniyev 2016; Matsiakh et al. 2018). In their literature survey, Mitchell et al. (2018) found a total of 132 fungi, 12 chromista (algae), 98 invertebrate and 44 lichens using *Buxus* species in the Caucasus and Europe. Of these, 43 fungi, 3 chromista and 18 invertebrate species have only been recorded on *Buxus* species and are at risk if *Buxus* spp. were disappearing.
2.15. 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?	minimal minor moderate major **massive**	low medium **high**	If no area-wide management method is implemented to lower populations in natural stands, e.g. through the introduction of a specific natural enemy from Asia, or if no resilience of *Buxus* stands are observed in the next few years, the risk is high that whole ecosystems will disappear, including many species that live exclusively in these ecosystems (Kenis et al. 2013; Mitchell et al. 2018).
2.16. 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?	minimal minor moderate **major** massive	low medium **high**	Several *Buxus* spp. stands are important components of protected sites in Europe. In Germany, the only sizeable *B. sempervirens* stand, which has now disappeared at more than 95%, was situated in the protected Natura 2000 reserve “Wälder bei Wyhlen“. The moth has damaged *B. sempervirens* stands in Natura 2000 sites in Italy (Raineri et al. 2017) and France (M. Kenis unpublished data) and is also present in the only Natura 2000 site hosting wild *B. sempervirens* in Belgium (T.

			Adriaens, pers. Comm.). In the EU Habitats Directive, *B. sempervirens* is listed as a characteristic species in five Annex 1 habitat types, including two that are priority habitat types: *Taxus baccata* woods of the British Isles and Mediterranean *Taxus baccata* woods (Mitchell et al. 2018).
2.17. 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?	minimal minor moderate **major** massive	low medium **high**	*Buxus balearica* is considered as "Near Threatened" in Spain, "Vulnerable" in Andalusia and it also occurs in the Balearic Islands. There is a single population in Sardinia (Di Domenico et al. 2012). We are not aware of *C. perspectalis* having reached *B. balearica* stands yet but field tests and observations in botanical gardens have shown that it is a suitable host for the moth (Brua 2014; Mitchell et al 2018). *Buxus sempervirens* is still abundant at European scale but some countries or regions have placed it in red lists, e.g. Luxemburg has classified it as "vulnerable" (Colling 2005) and the Alsace region in France as "Endangered" (Vangendt et al. 2014).
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	minimal minor moderate **major** massive	low **medium** high	The observed impacts of *C. perspectalis* on ecosystem services are caused the diminishing and/or disappearance of *Buxus* vegetation. In the Eastern Black Sea region, impacts on provisioning and regulating services have not yet been quantified but are likely (see 2.20). Mitchell et al. (2018) review the cultural services of *Buxus* trees in the Black Sea region, where wood and leaves are associated with different folklore and sacred rites since a very long time and are still important nowadays. In this region, the disappearance of one of the most important woody plants motivated international Actions, including from the FAO and the EU Office of the Special Representative for the South Caucasus and the crisis in Georgia (Mitchell et al. 2018).

			Box wood is also a very hard and highly valuable wood that is used for very specific purposes. For example it provides good sound projection because it is free from the grain produced by the growth rings. This makes it suitable for crafting high quality musical instruments such as the classical oboe and the violin (Savill 2013).
2.19. 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)?	minimal minor moderate **major** massive	low **medium** high	In the risk assessment area, impacts on provisioning and regulating services have not yet been quantified but are likely (see 2.20). In their review, Mitchell et al. (2018) also include cultural services of *Buxus* trees in the EU. While the cultural significance of the plant is probably less important than in other regions such as in the Eastern Black Sea region, *B. sempervirens* is nevertheless considered a plant of religious significance, in particular on Palm Sunday (Decocq et al. 2004). *Buxus sempervirens* is also a key component of many castles and historic gardens, which have to spray regularly to avoid the loss of these important cultural heritages. Box wood is also a very hard and highly valuable wood that is used for very specific purposes. For example it provides good sound projection because it is free from the grain produced by the growth rings. This makes it suitable for crafting high quality musical instruments such as the classical oboe and the violin (Savill 2013).
2.20. 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?	minimal minor moderate **major** massive	**low** medium high	The ecological disappearance of *Buxus* spp. stands is likely to have consequences on provisioning and regulating services. However, these consequences will depend on how the gaps within the forests are going to be filled by co-occurring species. A replacement by species that differ in structure or traits could affect various forest function (e.g. leaf litter decomposition rates and nutrient cycling), forest structure (e.g. taller trees establishing) and the forest community (e.g. changes in biodiversity) (Mitchell et al. 2018).

			So far, ecosystem processes and functions related to *Buxus* stands have been rather poorly studied in the EU. *Buxus sempervirens* is known to influence woodland succession by differentially influencing establishment and survival of tree species such as in the Pyrenees, where it favours *Fagus sylvatica* over *Abies alba* (Dolezal et al. 2004). *Buxus* spp. are also able to grow on steep crumbly slopes where they probably play an important role in sediment trapping (Duvigneaud 1969; Savill 2013). *Buxus sempervirens* traps 2.8 times more sediment than *Juniperus communis* and 1.5 times more sediment than *Pinus nigra*, but less sediment than *Lavandula angustifolia* (Burylo et al. 2012).
Economic impacts			
2.21. 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	minimal minor **moderate** major massive	low medium **high**	Economic costs of the invasion of *C. perspectalis* in other parts of its current area of distribution have not been calculated. In the Eastern Black Sea regions, non-EU European countries and in invaded ranges in East Asia (e.g, Northern China), most costs are probably borne by municipalities and private gardeners who have to spray or use other management methods to control the species or, when infestations are too heavy, replace their box trees by other plants. In the Eastern Black Sea Region, efforts to reduce the impact on the highly valuable natural *Buxus* stand (e.g. spraying, development of resistant cultivars, biological control programmes) have non-negligible costs although this has never been quantified.
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	minimal **minor** moderate major massive	low medium **high**	Economic costs of the invasion of *C. perspectalis* and related diminishing and/or disappearance of *Buxus* vegetation in the risk assessment area have not been calculated. When management costs are excluded, costs of damage and/or loss are probably minor. Some horticulturist specialised in Box tree production may be

			affected but, in general, the horticultural sector is probably not much affected by the loss of the *Buxus* spp. market since *Buxus* spp. are replaced by other species. Should management not be applied, there would be a risk for the historic gardens whose interest is partly based on topiary to lose tourists, but all of them protect their topiaries by spraying.
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	minimal **minor** moderate major massive	low medium **high**	Economic costs excluding management are likely to increase in the future if spread continues, but probably will remain minor.
2.24. 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)?	minimal minor **moderate** major massive	low **medium** high	In invaded areas in the risk assessment area, most costs are due to the use of pesticides, biologiocal control products or other management methods, including replacement by other plants and are probably borne by municipalities and private gardeners. However, there are no quantitative data on these costs available at EU or member state level.
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	minimal minor **moderate** major massive	low **medium** high	Since the organism has already reached most of its potential distribution in the risk assessment area, and ornamental box trees are disappearing from parks and gardens, economic costs probably remain moderate.
Social and human health impacts			
2.26. 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).	**minimal** minor moderate major massive	low medium **high**	In addition to impacts on cultural services (2.18 and 2.19), there are no other relevant impacts described.
2.27. 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.	**minimal** minor moderate major	low medium **high**	There is no indication that other relevant impacts will increase in the future.

	massive		
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	**minimal** minor moderate major massive	low medium **high**	*Cydalima perspectalis* is not known as food, host, symbiont or vector of other damaging organisms. It cannot be ruled out that it interacts with the numerous fungi that affect *Buxus* spp, including the invasive *Calonectria pseudonaviculata*, agent of box blight, but this has never been shown.
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	minimal minor moderate major massive	low medium high	N/a
2.30. 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?	minimal minor moderate **major** massive	**low** medium high	So far, parasitism by native parasitoids or pathogens is minimal in the risk assessment area (Wan et al. 2013; Belokobylskij and Gninenko 2016). Generalist predators such as wasps and birds are often observed praying on *C. perspectalis* larvae (Tunyiev 2016; M. Kenis, unpublished data), but their impact is unclear and they presently do not prevent total defoliation.

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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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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 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.

[10] Not to be confused with „no impact“.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.*. 2017)

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[11]	**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.*

[11] 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.

	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** 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*

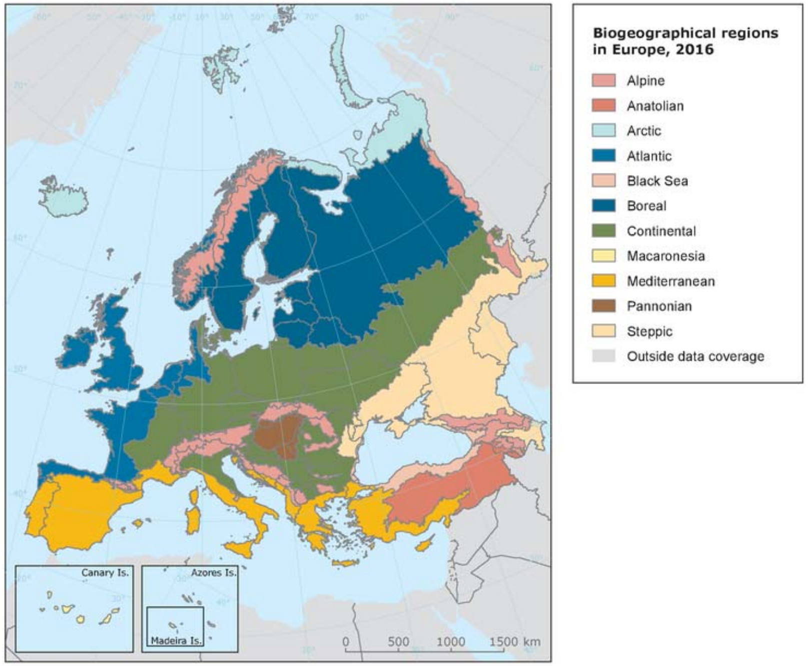
			composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living 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.*

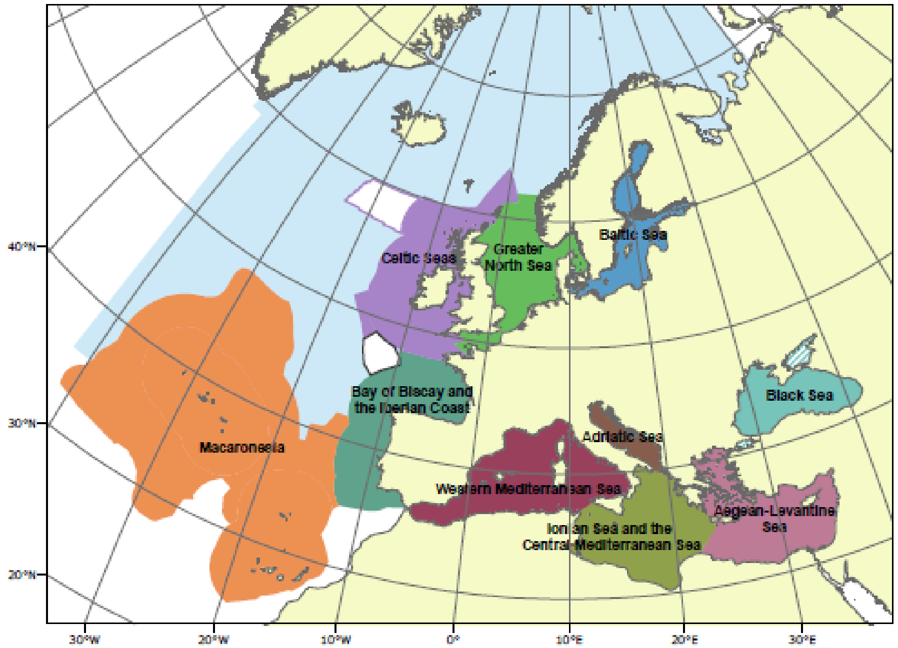
ANNEX V EU Biogeographic Regions and MSFD Subregions

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

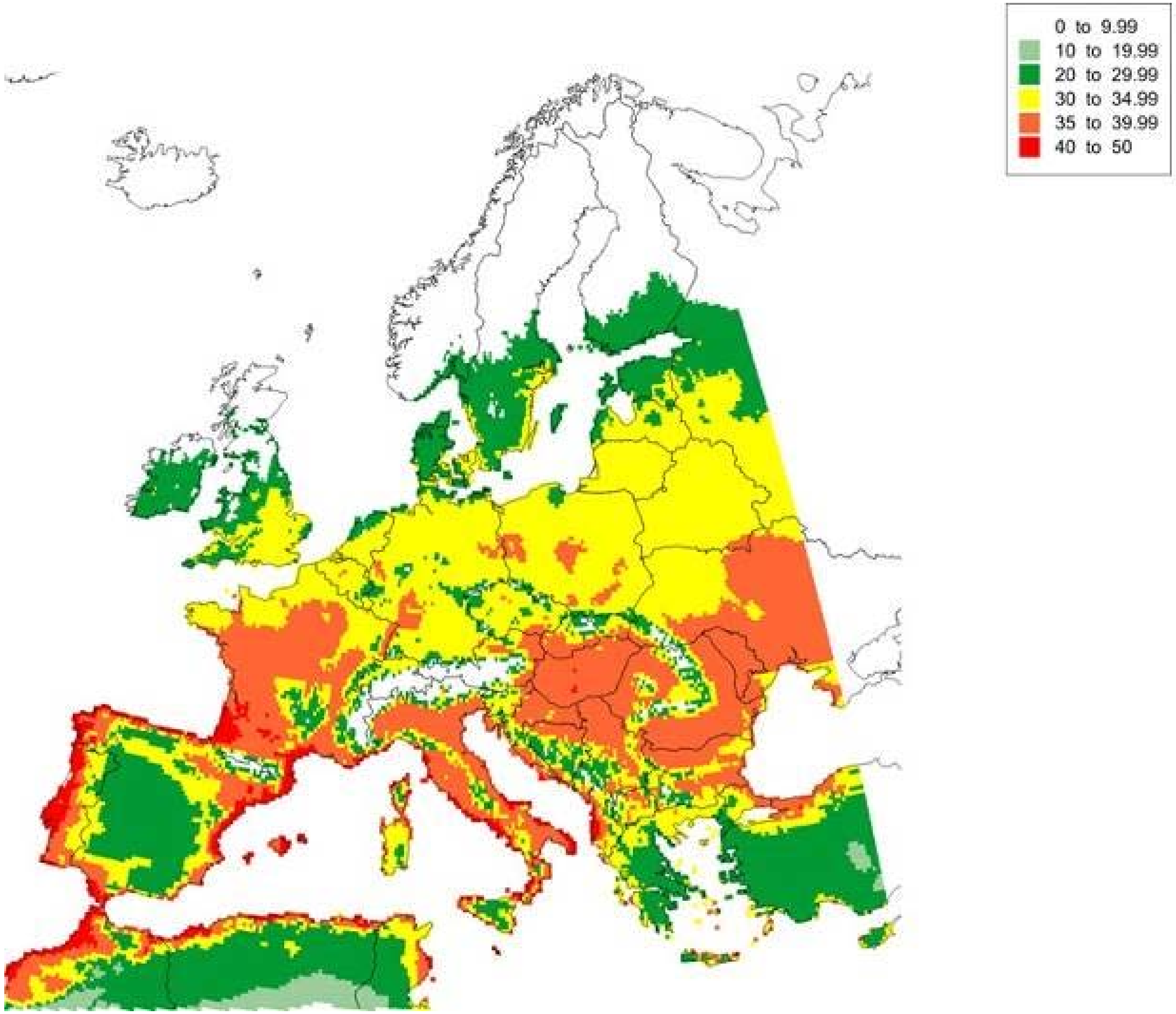
and

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

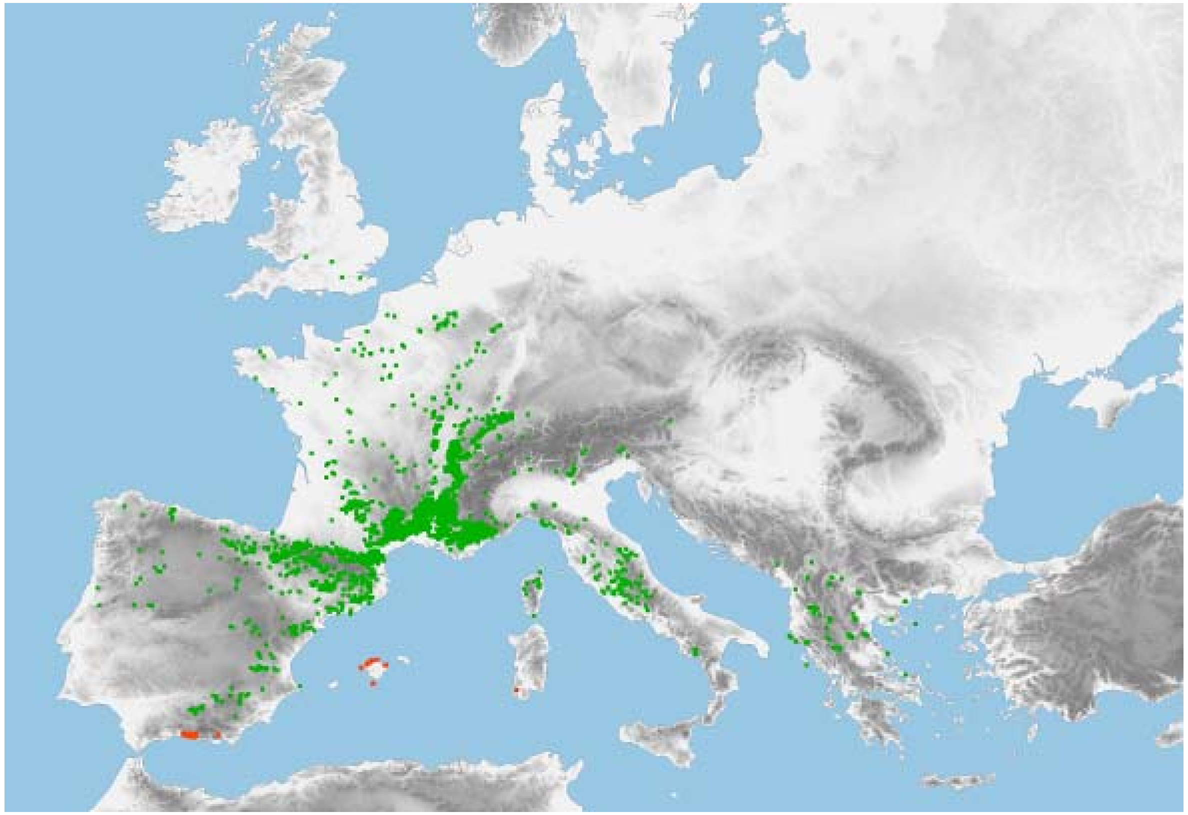




ANNEX I. Map of predicted distribution and relative abundance (Ecoclimatic Index) of *Cydalima perspectalis* in Europe. From Nacambo et al. (2014)



ANNEX 2. Map of occurrence of natural stands of *Buxus sempervirens* (green) and *B. balearica* (red) in Europe. From Di Domenico et al. (2012). With courtesy from F. Di Domenico



Template for Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	**Cydalima perspectalis (Walker, 1859)**
Species (common name)	**Box tree moth**
Author(s)	**Dr Marc Kenis, CABI, Switzerland**
Date Completed	**17.08.2018**
Reviewer	**Dr Peter Robertson, Newcastle University, UK** **Dr Gabor Vetek, Szent István University, Budapest, Hungary** **Dr Archie Murchie, Agrifood and Biosciences Institute, Northern Ireland, UK** **Dr Jørgen Eilenberg, University of Copenhagen, Denmark**

Summary

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.

It will be impossible to prevent the establishment of *C. perspectalis*, or eradicate newly established populations because the species is already widespread in Europe and the adult is a good flyer. Thus, it will sooner or later invade and establish in all suitable areas in the assessment area.

Damage by the species can be easily managed on ornamental box trees using pesticides or, preferably, biological control options such as *Bacillus thuringiensis* (Bt). Pheromones and light traps can also be used to monitor populations and enhance the efficacy of biological and chemical control. Other control methods such as the use of pheromones for mass trapping or mating disruption and releases of *Trichogramma* as an inundative biocontrol agent could be used to protect ornamental box trees, as well as the use of systemic insecticides by injection of highly valuable trees, but these methods still need further assessments. In contrast, the control of the moth on wild box stands is much more problematic. The use of insecticides is not allowed in forest areas in several countries and Bt cannot realistically be used to protect box tree stands over a long period. While, in the long term, the selection of resistant varieties and hybrids can be envisaged to repopulate forest stands, in the short and medium term only biological control can provide a solution. The adaptation of indigenous natural enemies to the invasive species cannot be ruled out but, given the urgency of the situation, the

introduction of natural enemies from Asia, in particular parasitoids, should be considered. In the short term, the rare populations of *Buxus balearica* in Mallorca should be protected by preventing the entry to the island, e.g. by regulating movements of *Buxus* spp. plants to the island. However, it is likely that, in the long term, the moth will be able to reach Mallorca by hitchhiking. It will be impossible to prevent the insect to reach the stands of *B. balearica* in Andalucía, where the moth will soon arrive, and in Sardinia, where the moth is already present, but these could be temporarily protected using Bt.

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	There is no method suitable for preventing the movement of *C. perspectalis* in continental Europe . It is already widespread in most of the assessment area and nothing can prevent its natural spread within continental Europe since it is a good flyer. European, regional and national plant protections organisations in Europe quickly gave up listing the species on their quarantine lists. For example, the European and Mediterranean Plant Protection Organisation (EPPO) removed *C. perspectalis* from its alert list in 2011 because of its wide distribution and unmanageable expansion (EPPO, 2012). Measures could be taken to prevent the entry of *C. perspectalis* in Mallorca, where the rare *Buxus balearica* (endemic to the Mediterranean coast) occurs. This could		High

	be done, e.g. by banning the transport of live *Buxus* spp. plants to the island. However, it is likely that, in the long term, the moth will be able to reach these islands by hitchhiking. Buxus balearica is also present in Sardinia but C. perspectalis is already present on the island on cultivated *Buxus* spp.		
Methods to achieve eradication	There is no method suitable for large-scale eradication since it is already widespread in most of the assessment area. Small-scale eradication can probably be achieved with a massive use of insecticides but the area would quickly be re-invaded from neighbouring areas.		High
Methods to achieve management	**Pesticides and biological control products**. Many insecticides can be used to control *C. perspectalis* in parks and gardens. Different insecticides with various active compounds are registered in different countries. Bacillus thuringiensis (Bt), a biological control agent based on a soil-dwelling bacterium, is very efficient and should be preferred over chemical insecticides to lower the impact on the environment and human health. Pheromone traps or light traps can be used to monitor adult flights and organise the spraying programme (Santi et al. 2015; Guérin et al. 2016). The injection of systemic insecticides to protect highly valuable trees is presently being studied (Bras et al.	Many pesticides and some biological control agents have shown to be very effective against *C. perspectalis* on ornamental box trees (e.g. Fora et al. 2016) and Bt is now regularly used in parks and gardens. Other biological control products, e.g. those based on entomopathogenic nematodes and botanicals like neem, are less effective than Bt (Göttig and Herz 2018). The cost of controlling *C. perspectalis* with Bt or chemicals in gardens and parks varies greatly between locations and countries. In Hungary, it was estimated that a control programme in a park including three treatments per year, each lasting two hours, costs approximately 400-500 Euro per year (G. Vetek, personal communication). However, the use of insecticides is not allowed in forest areas in several European countries and Bt cannot realistically be used to protect box tree stands over a long period. Bt could potentially be used to protect limited areas in highly valuable habitats until a sustainable classical biological control programme is implemented. For example, the stands of the threatened *Buxus balearica* in the Mediterranean biogeographical region could be temporarily protected using Bt.	High

	2017). Repellents are also being studied (e.g. Göttig and Herz 2017).		
	Mechanical control. In gardens with few small trees, eggs, larvae and pupae can be collected and destroyed by hand.		
	Classical biological control (CBC). CBC is the Introduction of a natural enemy of exotic origin to control an exotic organism, aiming at a permanent control of the target organism. Several parasitoid species are known to attack *C. perspectalis* in its area of origin (East Ssia), in particular the two braconids *Chelonus tabonus* and *Dolichogenidea stantoni*, which often reach high parasitism rates (Wan et al. 2014). Some of these Asian parasitoids could be introduced in Europe, provided they are sufficiently specific to avoid non-target effects on other insects.	No CBC programme has been implemented against *C. perspectalis* and, thus, no data on its effectiveness and cost-effectiveness are available. The implementation of a biological control programme would take some years, in particular for testing the specificity of the main parasitoids to prevent non-target effects. However, the method has proven to be cost-effective against many invasive insects (Cock et al. 2015), in particular forest pests (Kenis et al. 2017). In the case of *C. perspectalis* on wild box tree stands, It is not necessary to reach a full control of the pest but rather to lower populations until they can no longer kill trees. This level of control could probably be easily reached by the introduction of one or two parasitoid species from Asia. Based on comparisons with similar projects, a classical biocontrol programme including research and releases could be estimated at 300’000-400’000 Euro for a period of 4-6 years (M. Kenis, unpublished data).	Medium
	Other biological control agents. Various natural enemies that can be used in an integrated control approach have been studied but, although some are being promoted, they still need further assessments before being recommended in a large scale. They include, e.g. the releases of the egg parasitoids *Trichogramma* spp. (Göttig and Herz 2016; Guérin et al. 2016), or *Anagrapha falcifera* nucleopolyhedrovirus (AnfaNPV) (Rose et al. 2013).	*Trichogramma* parasitoids is sold in some countries but scientific evidences of their efficacy are needed. Other biocontrol agents are at the research stage.	Low

	Pheromones. Besides being a monitoring tool, pheromones can also be considered for mass trapping or mating disruption (Martin et al. 2015; Guérin et al. 2016),	Mass trapping has already been tried but with limited success (M. Kenis, unpublished observations from several sources). Mating disruption may be more efficient but would need extensive research for its development	Low
	Resistant varieties and hybrids could be considered for parks and gardens as well as for replanting destroyed box tree stands	The very slow growth of the plant will limit the prospects of this strategy in the short term but it may represent a long term solution	Medium

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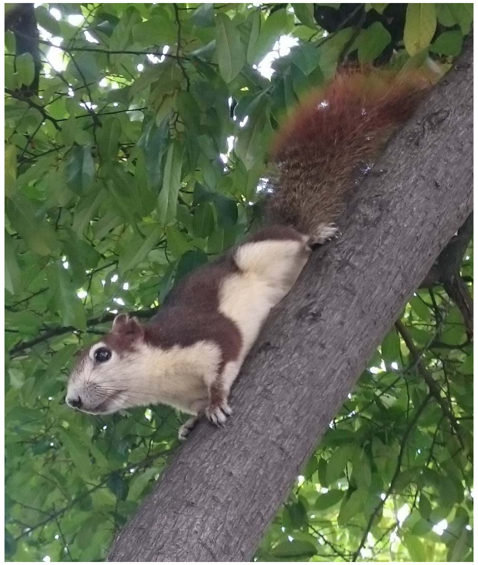
Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2[1]

Name of organism: *Callosciurus finlaysonii* (Horsfield, 1823)

EN: Finlayson's Squirrel, variable squirrel; IT: Scoiattolo di Finlaysoni; D: Finlayson-Hörnchen; F: Écureuil de Finlayson; NL: Finlaysoneekhoorn, finlaysonklappereekhoorn, Thailandeekhoorn, Thaise eekhoorn, variabele eekhoorn



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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

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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: Craig Shuttleworth, Bangor University, United Kingdom
Peer review 2: Sven Bacher, University of Fribourg, Switzerland
Peer review 3: Vinciane Schockert, University of Liège, Liège, Belgium

This risk assessment has been peer-reviewed by three independent experts and was discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study. Cover photo Tim Adriaens with permission.

Date of completion: 25 October 2018

Contents

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**[2]	**COMMENT**
Summarise Entry[3]	likely	medium	*C. finlaysonii* is already present in the risk assessment area after escapes and releases in Southern and Northern Italy. The primary pathway was release of captive animals in parks and woodlands. As the zoo and pet pathways are still active and the current populations represent a potential source of entry/translocation/natural dispersal to other parts of the RA area, the probability of entry is high. However, little information is available on the number of squirrels sold, kept as pets or kept in zoos.
Summarise Establishment[4]	very likely	high	The species is already established in Italy. It is adaptable and can thrive well in new areas when food and nesting places are available. In urban areas supplemental feeding is suspected to facilitate its establishment. *Callosciurus* squirrels are known to establish populations from few founders. The species distribution model predicts suitable areas for establishment in the Mediterranean, Continental and also Atlantic bioregion. This is corroborated by successful establishment of other tree squirrel species with a comparable native range. Moreover, *C. finlaysonii* is very tolerant to woodland degradation and fragmentation.
Summarise Spread[5]	moderately	medium	Quantitative studies on the sequential spread through suitable habitats and the possibilities of long distance colonization are not available for this species. However, the spread in southern Italy was rapid after an initial

[2] In a scale of low / medium / high, see Annex III

[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly

			lag-phase and the animals tripled their distribution range in four years, and increased their range 8.5 times in ten years. The total colonized area was 26 km^2 in 2005 but is currently estimated at 580 km^2. In case of new introductions in other countries spread could be moderate to large, depending on the habitat and landscape context. Humans translocations can promote the spread of the species.
Summarise Impact[6]	moderate	medium	In Italy the most evident damage caused by *C. finlaysonii* is bark stripping. Damage to ornamental trees or nurseries can be important, though this has not been quantified in economic terms so far. Bark stripping increases the risk of fungal infections and invertebrate damage, which ultimately can reduce timber yield. Damage to electric cables and other infrastructure by the species have also been reported. Data on impacts on native species and ecosystems are missing. However, impact can be inferred from other alien squirrel introductions in many European countries. Notably, interspecific competition with native species is likely as particularly, both *S. carolinesis* and *C. erythraeus* are already threatening red squirrel populations. The species is considered a predator of birds' nests in its native range, but no information is available for the introduced range. Transmission of pathogens could likely cause a risk but, currently, it is not documented. The potential impact on native such as the red squirrel or the endemic Calabrian black squirrel, woodland birds or dormouse is unknown but likely, especially considering impacts of other alien (tree) squirrels introduced and established in Europe.
Conclusion of the risk assessment[7]	High	medium	*Callosciurus finlaysonii* is already present in Italy and

[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

[7] In a scale of low / moderate / high

			the population in the South is rapidly expanding its range in recent years. The primary pathway for entry involves the escape or deliberate release of animals from captivity and the species is traded in Europe; therefore, new escapes or releases are likely. Climatic constraints do not seem to hamper successful establishment. The species profits from anthropogenically influenced landscapes and can establish from a limited number of founders. Damage through bark stripping can be considerable and impact on native species through competitive interactions is likely considering the impact of other exotic (tree) squirrels in Europe and the fact that *C.finlaysonii* now occur syntopic in the same habitat *S. vulgaris*. Confidence in the risk assessment is medium to high for establishment, spread and damage to forestry and plantations. Assessment of impact is medium confidence as data on the possible impacts on native species are absent, for the lack of specific studies, but are inferred from other squirrel species. The impacts of *C. finlaysonii* on native species and ecosystems should be better investigated. Also, the possible role of the species in disease transmission, with introduced individuals acting as vector or host of pathogens that can harm native wildlife (and potentially humans) represents a knowledge gap and should be investigated.

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Austria	–	–	–	–
Belgium	–	–	Y	–
Bulgaria	–	–	–	–
Croatia	–	–	Y	–
Cyprus	–	–	?	–
Czech Republic	–	–	–	–
Denmark	–	–	–	–
Estonia	–	–	–	–
Finland	–	–	–	–
France	–	–	Y	–
Germany	–	–	Y	–
Greece	–	–	Y	–
Hungary	–	–	–	–
Ireland	–	–	–	–
Italy	Y	Y	Y	Y
Latvia	–	–	–	–
Lithuania	–	–	–	–
Luxembourg	–	–	–	–
Malta	–	–	?	–
Netherlands	–	–	Y	–
Poland	–	–	–	–

Portugal	–	–	Y	–
Romania	–	–	–	–
Slovakia	–	–	–	–
Slovenia	–	–	–	–
Spain	–	–	Y	–
Sweden	–	–	–	–
United Kingdom	–	–	?	–

Biogeographical regions of the risk assessment area

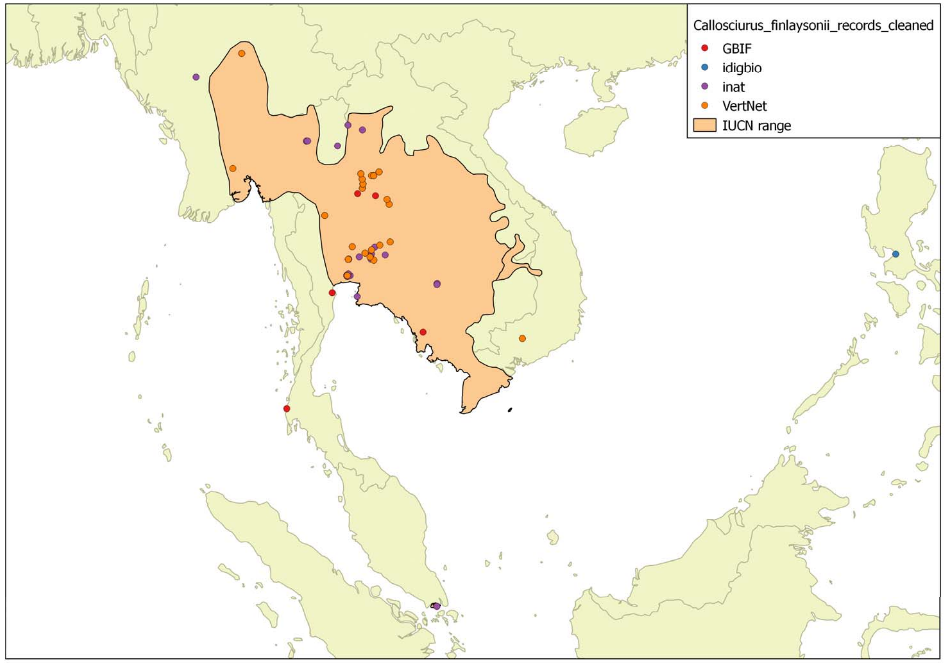
	Recorded	Established (currently)	Established (future)	Invasive (currently)
Alpine	–	–	–	–
Atlantic	–	–	Y	Y
Black Sea	–	–	Y	Y
Boreal	–	–	–	–
Continental	Y	Y	Y	Y
Mediterranean	Y	Y	Y	Y
Pannonian	–	–	–	–
Steppic	–	–	–	–

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Baltic Sea	NA	NA	NA	NA
Black Sea	NA	NA	NA	NA
North-east Atlantic Ocean	NA	NA	NA	NA
Bay of Biscay and the Iberian Coast	NA	NA	NA	NA
Celtic Sea	NA	NA	NA	NA
Greater North Sea	NA	NA	NA	NA
Mediterranean Sea	NA	NA	NA	NA
Adriatic Sea	NA	NA	NA	NA
Aegean-Levantine Sea	NA	NA	NA	NA
Ionian Sea and the Central Mediterranean Sea	NA	NA	NA	NA
Western Mediterranean Sea	NA	NA	NA	NA

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	• a list of the most common subspecies, lower taxa, varieties, breeds or hybrids This risk assessments deals with *Callosciurus finlaysonii* (Horsfield 1824) (Chordata, Mammalia, Rodentia, Sciuridae). The species can be adequately distinguished from other entities of the same genus. Common names: EN: Finlayson's Squirrel, variable squirrel; IT: Scoiattolo di Finlaysoni; D**:** Finlayson-Hörnchen; F: Écureuil de Finlayson; NL: Finlaysoneekhoorn, finlaysonklappereekhoorn, Thailandeekhoorn, Thaise eekhoorn, variabele eekhoorn Sixteen subspecies (nine mainland and seven island subspecies) have been reported (Lurz 2014), some of which have very restricted distributions (Corbet and Hill, 1992; Timmins and Duckworth 2008). There are several subspecies and yet to be classified forms in Thailand, Laos and Vietnam, some of which have localised ranges. A revision of the taxon is necessary to evaluate if one or more cryptic species are present (Duckworth 2017). The animals of the invasive populations in Italy have size and fur color similar to that described by Lekagul and McNeely (1988) for a population localized at Thonbury north of Ayutthaya (Thailand), which are smaller than other subpopulations of this species. The Thonbury population was included in the subspecies *C.* f. *bocourti* by Corbet and Hill (1992)
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 wild, in confinement or associated with a pathway of introduction]	The native Calabrian black squirrel *Sciurus meridionalis* is completely black with white belly and could easily be recognized from *C. finlaysonii* (Wauters et al. 2017). The absence of ear-tufts is a useful first guide to distinguish the species from the Eurasian red squirrel *Sciurus vulgaris*, which, however, could lose ear-tufts in summer. In Italy, typical colour morphs have a mostly olive brown back and cinnamon coloured tail, but this colour pattern shows a lot of variation (sometimes dark grey or brown back and/or tail, sometimes the tail underside or the entire tail is completely pale/white). Normally, there is a sharp line between the dark back and (yellow) white or isabel (pale grey-yellow) coloured belly (see also Mazzoglio et al. 2007). This fur coloration is different from the red to brown-black typical of *S. vulgaris*,

	but some confusion may arise in non-expert people. Coat colour in Finlayson's squirrels varies greatly between individuals and between subspecies/colour varieties within subspecies (see e.g. http://www.ecologyasia.com/verts/mammals/variable-squirrel.htm). Animals can range from all white, to all red, to all black. Due to the variability of the coat colour of this species, it is often referred to as variable squirrel (Bertolino, et al., 2000; Thorington, 2012). Therefore, animal traded may be different from those present in Italy. The most closely related species is Pallas' squirrel *Callosciurus erythraeus* (Pallas, 1779), which also has variable colour morphs (Boonkhaw et al. 2017). Timmins and Duckworth (2008) suggested that *C. finlaysonii* may hybridize with *C. erythraeus*. The hybridization between two species may more frequently happen in cage of pet stores. Similar species: Prevost squirrel *C. prevosti* can be distinguished from *C. finlaysonii* by its reddish-orange underparts and the whitish thighs and flanks in most subspecies. Grey-bellied Squirrel *C. caniceps* has a light grey or silvery belly, and in the dry season turns orange-brown above. Pallas' squirrel *C. erythraeus* is usually more brownish with a orange to reddish tint on the belly, and often with some black on the tail. Plantain Squirrel *C. notatus* is easily identified by the two cream and black stripes on the sides in combination with the orange belly. These cream and black stripes also occur in Black-banded Squirrel *C. nigrovittatus* but this species has a grey belly.
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	A risk assessment for the European Union was conducted for the Bern Convention (Bertolino 2015) using an adapted GB UK non-native organism risk assessment (NNRA) scheme version 3.3. The final evaluation was: risk of entry: 4 (very likely), risk of establishment: 4 (very likely), risk of spread: 2 (moderate), impacts: 2 (moderate). Furthermore, Dijkstra and Dekker (2008) made a risk assessment for several species of exotic squirrels in the Netherlands. They mentioned trade and keeping of *C. finlaysonii* (subspecies *bocourti* and *ferrugineus*) was relatively limited in The Netherlands but did not assess the risk associated with their introduction. There are no mention of subsequent observations of *C. finlaysonii* in the wild (Dijkstra & Dekker 2013). In Belgium, an impact assessment was performed using the Invasive Species Ecological Impact Assessment (ISEIA) protocol guidelines (Branquart 2007, Branquart et al. 2009; Vanderhoeven et al. 2015). In Belgium the species was categorized as an alert list species with the ecological impact assessment protocol ISEIA (http://ias.biodiversity.be/species/show/127) (11 out of maximum score of 12), as it scored high on establishment potential and dispersal into natural habitats. In Germany, the species was assessed in the Grey List of potentially invasive species (Rabitsch et al. 2013). The results of the present study, which in fact builds on the risk assessment made for the Bern Convention by Bertolino (2015), are fully consistent with the assessments mentioned above.
A4. Where is the organism native?	The species is native to South East Asia, from central Myanmar, Thailand, Laos, Cambodia to Vietnam

<table>
<tr><td></td><td>(Moore & Tate 1965; Wilson & Reeder 2005; Duckworth et al. 2008). Many subspecies only occur on isolated islands. Of the 16 subspecies, 12 are distributed in Thailand, making this the main distribution area (Boonkhaw et al. 2017).


Native range of C. finlaysonii (Duckworth et al. 2008) showing selection of records used for the species distribution model (see ANNEX VI - Species Distribution Model).</td></tr>
<tr><td>A5. What is the global non-native distribution of the organism outside the risk assessment area?</td><td>Callosciurus finlaysonii was introduced to Singapore and the Philippines (Bertolino & Lurz 2013). Some of the animals present at Hamamatsu (Japan), previously considered to be C. erythraeus, in fact carried mtDNA of C. finlaysonii (Oshida et al. 2007). The introduction of C. finlaysonii to Japan was confirmed by further work by Kuramoto et al. (2012).</td></tr>
<tr><td>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?</td><td>The species has been reported and is established in the Continental and Mediterranean bioregions.</td></tr>
<tr><td>A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current</td><td>Under current climatic conditions the Mediterranean bioregion is predicted by the species distribution model to be suitable for establishment. Under moderate (RCP4.5) and extreme (RCP8.5) emission scenarios, by 2070, the potential area for establishment is predicted to increase with the Atlantic,</td></tr>
</table>

climate and under foreseeable climate change?	Continental and Black sea regions becoming suitable. It should be noted, however, that all current European records of the species are outside climatic boundaries of its subtropical native range (see Annex VI), indicating an adaptability of the species probably not fully captured by the model. Based on the species distribution model (Annex VI), the most limiting factors for establishment in northern part of Europe are cold winters. In the Mediterranean, according to the model, the main limiting factor is precipitation (drought). The species distribution model assumed areas which were colder and drier than the current occurrences were unsuitable for the species i.e. areas with a mean temperature of the warmest quarter (Bio10) below 19°C or with a minimum temperature of the coldest month (Bio6) below -1°C or a minimum annual precipitation (Bio12) of less than 600mm per year. There are considerable uncertainties around model predictions due to limited information on the species eco-physiological requirements and the known adaptability of the species to climatic conditions different from its subtropical native range . As the squirrels are mobile and the species could adapt, it can be expected that it could colonise areas predicted as unsuitable. For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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 species has only been recorded in Italy where populations are established. Two populations exist in northern and southern Italy. In the North, the species is established in and around an urban park within the city of Acqui Terme (Bertolino et al. 1999), following the release of two pairs in 1981 (Bertolino & Lurz 2013). In the South, *C. finlaysonii* was introduced in the mid-1980s through a release of 3-4 pairs (Aloise & Bertolino 2005). Initially, it remained restricted to an urban area, but after this initial lag-phase it later rapidly spread along the Tyrrhenian coast in both directions (south and north) along an area that stretched over 19 km of coastline in 2004 (Aloise & Bertolino 2005). This increased to 45 km in 2004 (Aloise & Bertolino 2008; Aloise et al. 2010). The total colonized area was 26 km^2 in 2005 (Aloise & Bertolino 2005) and increased to about 68 km^2 in 2008 (Aloise et al. 2010). Currently (2018), the area of occupancy in Italy is estimated at 580 km^2 based on a minimum convex polygon around known records (Bertolino & Di Febbraro unpublished data). It occurs at a maximum altitude of 841 m a.s.l.

	 Current (2018) distribution of *C. finlaysonii* in Italy.
A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	Under current climatic conditions a number of Mediterranean EU Member States are predicted to be suitable for establishment: Italy, Spain, Croatia and Greece, potentially also Malta and Cyprus. Under a moderate (RCP4.5) emission scenario, by 2070, the potential area for establishment is predicted to increase with a number of EU Member States in the Atlantic and Continental bioregion such as Portugal and France. In an extreme (RCP8.5) emission scenario large parts of northwest Europe in Belgium, The Netherlands, Germany and the southern part of Great Britain are predicted as suitable for the species establishment. See Question A7 or Annex VI for more details on the distribution model.
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?	Data on the ecological impact of the species are scarce and scientific studies are still lacking. *Callosciurus finlaysonii* is considered a frequent predator of bird eggs in its native range (Bertolino & Lurz 2013). Data on damage are known only from Italy in the risk assessment area (Bertolino et al. 2004; Mori et al. 2016).
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	Mediterranean, Continental
A12. In which EU member states has the species	Italy

shown signs of invasiveness?	
A13. Describe any known socio-economic benefits of the organism.	The species is kept and traded as a pet thus represents aesthetic and economic values. It is possibly also kept in zoos, wildlife parks, animal rehabilitation centres and private collections, but it is probably an uncommon species (see question 1.4b). Animals are usually on display for a price of about 50 euros. More information on trade can be found in question 1.4a.

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."
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment	few	medium	Active pathways include escapes from zoos, (private) wildlife collections, pet shops etc. and the release of (pet) animals into the environment. Human assistance may amplify the potential of the species spread after first introduction as is illustrated by at least two

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

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

section)			reported translocations (i.e. deliberate capture, transportation and release of animals, Aloise & Bertolino 2005; Aloise et al. 2011).
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	Release in nature – Landscape/flora/ fauna "improvement" in the wild Escape from confinement – Botanical garden/zoo/aquaria		
Pathway name:	**Release in nature – Landscape/flora/ fauna "improvement" in the wild**		
1.3a. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional	high	The species has been intentionally released in nature or parks for aesthetic reasons. This has been the main pathway of *C. finlaysonii* introductions in Italy (Bertolino et al. 1999; Aloise & Bertolino 2005). Squirrels are often released in or near urban areas such as parks, where they benefit from supplementary feeding.
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	moderately likely	low	In the absence of trade statistics, an internet survey was conducted between 17-21 January 2015, in order to investigate whether live *C. finlaysonii* appear to be traded within the EU, and whether there appears to be demand for these species as pets. The procedure was similar to the one used by UNEP-WCMC (2010) for *C. erythraeus* and *S. niger*. Adverts for the sale of *C. finlaysonii* were found on websites from Spain, Italy, Germany and The Netherlands. There were several advertisements for people wanting squirrels in general and also looking specifically for *C. finlaysonii*. Considering the inclusion of other exotic squirrel species (*C. erythraeus*, *S. carolinensis*, *S. niger*) in Regulation (EU) 1143/2014 which ban those species

			from the pet-trade, there is the possibility that the trade of *C. finlaysonii* could increase in the future.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	high	The species is often released in urban and suburban parks, which provides suitable habitat with supplemental feeding which can increase survival in the initial establishment phase (Bertolino et al. 1999; Aloise & Bertolino 2005; Bertolino & Lurz 2013). From here the species can spread to more natural habitats (Aloise & Bertolino 2005).
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	likely	high	This pathway includes deliberate releases by pet owners which are the result of intentional introduction. The species is already present in Italy and is traded in other European countries. The main pathway of *C. finlaysonii* introductions in Italy has been releases of pet animals (Bertolino et al. 1999; Aloise & Bertolino 2005). Human assistance may amplify the potential of the species spread as is illustrated by at least two reported translocations (Aloise & Bertolino 2005; Aloise et al. 2011). These squirrels are appealing to humans, which can promote the spread of the species with translocation from one area to another. This is exemplified by *C. finlaysonii* in Southern Italy (Aloise & Bertolino 2005; Aloise et al. 2011), *C. erythraeus* in Argentina (Guichón et al. 2005, 2015) and Japan (Miyamoto et al. 2004), *S. carolinensis* in Italy (Martinoli et al. 2010; Signorile et al. 2016) and UK (Shorten 1954; Signorile et al. 2016), and with *S. stramineus* in Perù (Jessen et al. 2010). Translocations potentially create new propagules and could help the species to overcome geographical or ecological barriers, and increase the spread rate. The deliberate release of animals from captivity (see as a pathway example the video on YouTube regarding an illegal release of a chipmunk (http://www.youtube.com/watch?v=p_Ee4Bvk-eU) is probably the primary pathway of entry. As long as the

			species continues to be kept in captivity and is sold by pet shops the probability of releases remains (Bertolino 2009; d'Ovidio et al. 2014; see point 1.4a). On top of that, established wild populations could be the source of animals for new introductions (Aloise & Bertolino 2005; Aloise et al. 2011) or for an illegal trade of the species (Signorile et al. 2016).
Pathway name:	**Escape from confinement – Botanical garden/zoo/aquaria**		
1.3b. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	unintentional	high	This pathway refers to escaped *C. finlaysonii* from facilities such as zoological gardens where wild animals are confined within enclosures (or live in semi-wild conditions), are displayed to the public, and in which they may also breed. It is different from the pet pathway in so far as the animals are typically on display to the general public (IUCN 2017). *Callosciurus finlaysonii* is arboreal and thus a good climber. It will be able to escape from a damaged or inadequately secured enclosure, as has been the case with other species of tree squirrels (*C. erythraeus*) and red squirrel *S. vulgaris* in Europe (e.g. Shuttleworth et al. 2014; Adriaens et al. 2015; Dijkstra & La Haye 2017).
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	unlikely	Medium	Whilst there is no data available on the total 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 exceptions of Cyprus and Malta). The information provided by EAZA (EAZA, 2018 personal communications) indicates the species is not kept by EAZA Member zoos/aquariums. In France *C. finlaysonii* was censused in only one zoo near Bordeaux (https://www.fermeexotique.fr/details-ecureuil+de+finlayson-167.html) that is not affiliated to AFDPZ (Association Francaise Des Parcs Zoologiques)

			(personal communication J.-F. Maillard, survey ONCFS 2017). According to http://www.zootierliste.de/ the species is at least on display in the Netherlands, France, Russia and the UK. Unintentional escapes of native red squirrels *S. vulgaris* from woodland enclosures have been documented (e.g. after storm damage to the enclosures) indicating squirrel escapes are likely to happen despite the animals being properly housed (Shuttleworth et al. 2014).
1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	moderately likely	medium	
1.6b. How likely is the organism to survive existing management practices during passage along the pathway?	likely	medium	
1.7b. How likely is the organism to enter the risk assessment area undetected?	moderately likely	medium	The species could be confused by people without experience with the native Eurasian red squirrel and therefore not be reported (see A2), Specific surveillance (e.g. wildlife camera trapping networks, surveillance with hair tubes) is largely lacking.
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	low	
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Likely	medium	Zoos are often located in urban areas and similar to the pathway above, it is likely that escaped animals survive in parks, gardens etc.
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	Unlikely	low	Probably escapes happen but such events are rather rare as keeping the animals captive is in the interest of the zoos. However, Shuttleworth et al. (2014) report escapes of red squirrels *S. vulgaris* from mesh wire woodland encloruses in a captive Zoological red squirrel collection, confirming that escapes are always

			possible even when squirrels are properly housed. Also, populations of the congeneric species *C. erythraeus* in the Netherlands, Belgium, Italy and France originated from escaped animals (e.g. Adriaens et al. 2015; Dijkstra & La Haye 2017). A low confidence has been given because of lack of information as to how many animals are actually kept in the RA area which makes it difficult to estimate the overall likelihood.
Pathway name:	**Escape from confinement –Pet / aquarium / terrarium species (including live food for such species)**		
1.3c. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	unintentional	high	This pathway refers to escaped *C. finlaysonii* from confinement or from controlled environments where they were kept by private collectors or hobbyists for recreation, enjoyment, companionship and/or trading (e.g. breeding/cultivation for sale to other collectors) (IUCN 2017). It also includes escapes from pet shops.
1.4c. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	Likely	medium	The species is present in trade and low numbers of animals can already represent a risk of restablishment (see Q1.24).
1.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	Moderately likely	medium	
1.6c. How likely is the organism to survive existing management practices during passage along the pathway?	Likely	medium	
1.7c. How likely is the organism to enter the risk assessment area undetected?	Likely	medium	The species could be confused by people without experience with the native Eurasian red squirrel and therefore not be reported (see A2). Specific surveillance

			(e.g. wildlife camera trapping networks, surveillance with hair tubes) for squirrels is largely lacking.
1.8c. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	low	
1.9c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Likely	medium	
1.10c. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	Possible	low	
End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	Likely	high	See comments 1.10a and 1.10b
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	Likely	high	Climate change is not expected to influence the likelihood of entry into the RA area, which therefore remains likely.

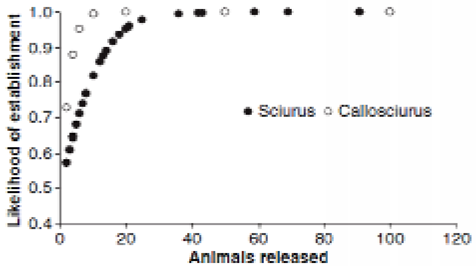
PROBABILITY OF ESTABLISHMENT

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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	moderately likely	medium	The species distribution model predicts suitable areas for establishment outside the current Italian range elsewhere in the Mediterranean based on several climatic variables. However, propagule pressure and human influence (e.g. supplemental feeding, urbanization, forest fragmentation) are expected to contribute to this.
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	likely	high	In Europe the species is adapted to Mediterranean deciduous forests and Mediterranean pine forests and to urban and suburban areas (Bertolino et al. 2004; Aloise and Bertolino 2005; Rima et al., 2007). It feeds opportunistically and seasonally, mainly on plant matter, i.e. seeds, fruits, buds, flowers and sap, occasionally animal food including insects and bird eggs/nestlings.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	widespread	high	Suitable habitats (Mediterranean deciduous forests, Mediterranean pine forests, urban and suburban areas) are present and widely distributed in Southern Europe.
1.16. 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 ?	NA	NA	No other species is vital for the species survival, development or reproduction.
1.17. How likely is it that establishment will occur despite	likely	high	Competition for natural resources with existing

competition from existing species in the risk assessment area?			species will not limit the establishment in the risk assessment area.
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very likely	medium	Predators, parasites and pathogens present in Italy did not hinder the establishment of the species (Aloise & Bertolino 2005; Aloise et al. 2011).
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very likely	high	The species is very tolerant to forest fragmentation or woodland degradation, as observed in its native range (Duckworth et al. 2008).
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	likely	high	The species is adaptable and can profit from urbanisation (supplemental feeding). As mentioned above, forest fragmentation could favour the establishment of the species.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	likely	medium	Finalsyson's squirrels have been removed with live-traps from a small area in Southern Italy (Ricciardi et al 2013), but no details of the effects on the population are available. Experiences with other alien squirrels show that high removal rates are necessary to obtain success and that numbers return quickly to pre-control levels once killing is stopped e.g. where culling was localised and undisturbed adjacent populations were in close proximity in connected habitat patches (Lawton & Rochford 2007). Once established, squirrels are difficult if not impossible (with large populations) to eradicate though some success can be achieved at a local level with a high control effort (Schuchert et al. 2014). For instance, the grey squirrel was eradicated form an island of the size of the *C. finlaysonii* range in southern Italy (Schuchert et al. 2014).
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	likely	high	The species may establish from a very limited number of founders. As an illustration, established populations in Europe, Singapore and Japan

			originated from few animals (Bertolino 2009), thus proving the adaptability of *C. finalysonii* to new habitats. Females can have two to three litters per year with 1-4 weaned young; varying percentage of adult females reproduce in a given season.
1.23. How likely is the adaptability of the organism to facilitate its establishment?	likely	high	Tree squirrels are considered particularly adaptable because of their relatively high reproductive potential, wide dietary range, and plasticity to anthropogenic habitats (Palmer et al. 2007, UNEP-WCMC 2010). In its native range it occurs in many habitats from primary and secondary forests to open woodland and plantations (Lurz 2014). It is very tolerant to woodland degradation and fragmentation (Duckworth et al. 2008). It is adaptable in its diet and habitat requirements
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very likely	high	Tree squirrels are known to form viable populations from very few founders. The likelihood ratio for a pair of *Callosciurus* spp. (*C. erythraeus and C. finlaysonii* were considered) to successfully establish a viable population is 73% and a likelihood ratio of 90% is achieved from as little as 4 animals (Wood et al.2007; Bertolino 2009).  Likelihood of *Sciurus* and *Callosciurus* establishment as a function of the number of

			animals released (Bertolino et al. 2009)
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	very likely	high	The species has successfully established in Singapore and Japan and it is very likely to establish in the risk assessment area beyond the current Italian range.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	likely	low	The likelihood of this to happen would depend on the number of animals released/escaped. However, such non-reproducing animals in the wild are quite common in other squirrel species in Europe (high number of casual sightings of species like *Tamiasciurus hudsonicus*, *Callosciurus prevosti* etc.).
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	likely	medium	*Callosciurus finlaysonii* originates from tropical and subtropical broadleaf forests in Asia. They have colonized the Continental bioregion in Northern Italy (Bertolino & Lurz 2013). This is supported by the species distribution model. Under current climatic conditions the Mediterranean bioregion is predicted to be suitable for establishment. Based on the species distribution model, the most limiting factors for establishment in the northern part of Europe are cold winters. In the Mediterranean, the main limiting factor is precipitation (drought). These factors could reflect thermal stress in the active/breeding season. The species distribution model assumed areas which were colder and drier than the current occurrences were unsuitable for the species i.e. areas with a mean temperature of the warmest quarter (Bio10) below 19°C or with a minimum temperature of the coldest month (Bio6) below -1°C or a minimum annual precipitation (Bio12) of less than 600mm per year. However, there are uncertainties around model

			predictions due to limited information on the species eco-physiological requirements and the adaptability of the species, i.e. all current European records of the species are outside climatic boundaries of its subtropical native range (see Annex VI), indicating an adaptability of the species not fully captured by the model. As the squirrels are mobile and the species is quite adaptive, it can be expected that it could also colonise areas predicted as unsuitable by the model. As an illustration, all current European records of the species are outside climatic boundaries of its subtropical native range. Besides climatic constraints, propagule pressure and human influence can also play a role in establishment success.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	likely	medium	Under moderate (RCP4.5) and extreme (RCP8.5) emission scenarios, by 2070, the potential area for establishment is predicted to increase to more northern regions with the Atlantic, Continental and Black sea region also becoming suitable. For more details on the SDM see questions A7, A9 or Annex VI.

PROBABILITY OF SPREAD			
Important notes: • 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	high	medium	In southern Italy *C. finlaysonii* has spread over an area of 581 km^2, after a lag-phase of more than 20 years. The dispersal capacity of *C. finlaysonii* seems to be high, mainly of immature individuals, which will colonize new areas. The species has spread along the Tyrrhenian coast in a few years in Mediterranean deciduous and pine forests and to urban and suburban areas (Bertolino et al. 2004; Aloise and Bertolino 2005; Rima et al., 2007).
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	low	medium	
2.2a. 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 2.3 to 2.9 (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. 2.3a,	Unaided - Natural dispersal across borders		Studies of dispersal distances are not available for this squirrel species. The only data available relates to the spread in southern Italy where the species tripled its area of occupancy in four years and increased it by 8.5 times in ten years. The population in Southern Italy is rapidly spreading along the Tyrrhenian coast, having colonized an area of 26 km^2 by 2004 (Aloise & Bertolino 2005). Its range increased to 68 km^2 in

2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.			2008 (Aloise & Bertolino 2008; Aloise et al. 2011) and up to 581 km^2 by 2018. The population in Northern Italy is still present after thirty years since its initial introduction, though localised in an urban area and surroundings. In Singapore, the species is slowly spreading in the city (Benjamin Lee pers. comm.). In case of newly established populations in other countries, the spread rate could be from moderate to high, depending on the habitat.
Pathway name:	**Unaided - Natural dispersal across borders**		
2.3. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	unintentional	high	Unaided natural dispersal across borders is unintentional
2.4. 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?	moderately likely	low	See 2.2a
2.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply alonsouthern italyg the pathway.	very likely	high	See 2.2a Active dispersal, mainly of immature individuals, which will colonize new areas of suitable habitat.
2.6. How likely is the organism to survive existing management practices during spread?	very likely	high	The species is spreading rapidly in Southern Italy, and remains present in Northern Italy after 30 years. The population in Singapore is steadily increasing.
2.7. How likely is the organism to spread in the risk assessment area undetected?	moderately likely	low	The species is not easily recognisable from the naive Eurasian red squirrel and specific surveillance (e.g. wildlife camera trapping networks, surveillance with hair tubes) is largely lacking. Therefore, local spread may be undetected.
2.8. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	likely	medium	*Callosciurus finlaysonii* is an adaptable species, occupying several types of forest, even fragmented or degraded forest, and urban areas, such as parks. It

			could also benefit from supplementary feeding in urban areas.
2.9. Estimate the potential rate of spread within the Union based on this pathway (please provide quantitative data where possible)?	moderately	medium	Even though spread in Southern Italy is happening at a fast rate (see Q 2.11), this is not the case for the population in the north of the country. The latter population, is still concentrated within an urban area thirty years after its initial introduction. The lack of spread may be related to the introduction of only two pairs (3-4 pairs in the South) and/or to the difficulty to adapt to the habitats outside the city, where supplemental feeding by humans is lacking.
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	difficult	medium	A control program was activated in Southern Italy, but results are not available yet (Ricciardi et al. 2013). From experience gained in Europe with other alien squirrels, the species could probably be contained where it does not spread over large areas, partly because of seasonally high trappability, and partly because of easy recognition of the species in new areas. However, practical difficulties are likely to arise because of diverse landownership patterns in control areas with possible difficulties in access private property and because of potential public opposition to control/eradication (Barr et al. 2002; Rushton et al. 2002; Anonymous 2013).
2.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues and provide quantitative data where possible).	moderately	medium	Studies of dispersal distances are not available for this squirrel species. The only data available relates to the spread in southern Italy where the species tripled its area of occupancy in four years and increase it by 8.5 times in ten years. See also 2.2a
2.12. Estimate the overall potential rate ofspread in relevant biogeographical regions in foreseeable climate change conditions *(please provide quantitative data where possible)*	rapidly	low	

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is 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?	minor	low	Data on the ecological impact of *C. finlaysonii* are scarce and proper studies are lacking. The species is considered a frequent predator of bird nests in its native range (Bertolino & Lurz 2013), but there is no information for the introduced range available. In its native range, *C. finlaysonii* is considered an important seed consumer and seed dispersal agent (Kitamura et al. 2004, Chanthorn et al. 2007, Suzuki et al. 2007).
2.14. 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)?	moderate	low	The activity of bark stripping typical of the species increases the risk of fungal infections and invertebrate damage on trees which can indirectly have an influence on associated woodland biota. There are some potential problems of predation on bird eggs/nestlings, but studies in the risk assessment area are missing and also from its native range there are only qualitative data available hence low confidence (Bertolino & Lurz 2013). Transmission of pathogens could likely cause a risk but there are no data available. Introduced

			populations of the related Pallas's squirrel in Europe were shown to host a number of co-introduced macroparasites such as lice and nematodes but these pathogen guilds were dominated by a few specialist taxa imported with the founders (Dozières et al. 2010). Dozières et al. (2010) consider those as minimal sanitary risks for both native fauna and humans in urbanized habitats where the animals were sampled.
2.15. 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?	moderate	low	The potential impact on other species such as the red squirrel *S. vulgaris* and the Calabrian black squirrel *S. meridionalis*, woodland birds or dormouse (Gliridae) is unknown but possible, considering impacts by other alien squirrels introduced in Europe (i.e. *S. carolinensis*, *Tamias sibiricus*, *C. erythraeus*). Particularly, both *S. carolinensis* and *C. erythraeus* are already threatening local red squirrel populations through interspecific competition and disease transmission (Gurnell et al. 2004; Shuttleworth et al. 2016; Mazzamuto et al. 2017a,b). If bark stripping produced significant damage frequently, this could influence woodland management practices, with a shift away from trees susceptible to squirrel damage (Mayle 2005) (See 2.22), and with an impact on the flora and fauna associated with specific woodland types.
2.16. 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?	NA	NA	Current impact of *C. finlaysonii* on conservation assets is undocumented.
2.17. 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?	moderate	low	Although not included in the Habitat's Directive, the possible interference with the native red squirrel and the Calabrian black squirrel could decrease the conservation status of these species as well as many areas where they occur. Calabrian black squirrel, which was recently promoted to species level based on genetic studies, has a small range in the RA area and is endemic

			to the Calabrian mountains of southwestern Italy (Wauters et al. 2017). The region is potentially suitable for establishment of *C. finlaysonii* and therefore the species could be impacted by this invader although it would probably not disappear entirely (hence moderate impact).
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	minimal	low	This has not been assessed in Singapore and Japan, although it can be assumed that this impact is minimal, especially in Singapore, given the small size of the population.
2.19. 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)?	moderate	medium	The observed impacts of *C. finlaysonii* on ecosystem services are caused by bark stripping and seed dispersal. Bark stripping by *C. finlaysonii* has been observed in Italy (Bertolino et al. 2004, Aloise & Bertolino 2005: Mori et al. 2015). This can cause (secondary) infections in trees and has already led to the phytosanitary cutting of ornamental trees in Northern Italy (see 2.22). The species is also a seed disperser and could be a vector or host for pathogens. In natural forests, this could influence forest structure, species composition, the amount of (standing) dead wood, forest management practices etc. Given the above, impacts could occur on the following ecosystem services: provisioning – biomass – cultivated terrestrial plants, provisioning – biomass – wild plants, regulating services – regulation of physical, chemical, biological conditions - lifecycle maintenance (e.g. seed dispersal) and pest and disease control, as well as cultural – experiental interactions (due to phytosanitary cutting in urban green areas). These effects are probably rather local and reversible hence moderate impact.
2.20. How important is the impact of the organism on	major	low	Widespread but reversible impacts on several

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?			provisioning and regulating services in the future are possible if the species spreads in the risk assessment area (see 2.19).
Economic impacts			
2.21. 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	NA	NA	No information available
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	moderate	medium	In Italy the most evident damage caused by this species is bark stripping, which can cause (secondary) infections in trees and warrant phytosanitary cutting of 42 out of 308 ornamental deciduous trees. Damage can be considerable, yet is not quantified in economic terms. In Northern Italy, squirrels were observed eating plant matter, including bark and sap, seeds and fruits, buds and flowers; animal food included insects and insect honeydew. Bark stripping damage has been estimated to occur on 80% of the trees (11 species of deciduous and coniferous trees) in an urban park in the population in Northern Italy (Bertolino et al. 2004, Aloise & Bertolino 2005: Mori et al. 2015). In southern Italy, it has been estimated at a mean of 40% for nine wooded areas (Mori et al. 2016). Damage to electric cables and other infrastructure has also been reported (Aloise & Bertolino 2005; Aloise et al. 2011).
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	major	low	The damage through bark stripping and cable gnawing would be major if the species were not eradicated and was able to extend its range and invade other suitable areas in the RA area. Since quantified data on economic cost are not available confidence is low.
2.24. 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)?	moderate	low	Considering previous management programs on other squirrel species (e.g. grey squirrel, Pallas's squirrel) costs could be high (see question 2.25).

2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	major	medium	Considering previous management programs on other squirrel species (e.g. grey squirrel, Pallas's squirrel) future control costs could be considerable. This can be inferred from control actions on the related Pallas's squirrel *C. erythraeus* in the RA area. Even rapid eradication from Belgium which included the permanent removal of 250 animals within a 5-year timeframe came at a considerable 200k€ cost including the cost of surveillance and post-eradication monitoring (Adriaens et al. 2015). A Pallas's squirrel invasion in The Netherlands (Weert) was tackled by rapid eradication at a cost of 330k€ to run the programme and remove 250 squirrels, but with the help of volunteers (pers. comm. M. La Haye; Dijkstra & Bekker 2012; Dijkstra 2013a,b). Cost are higher for established populations with a higher number of animals, such as in France where control actions for Pallas's squirrel were planned at about 100k€ per annum for the period 2011-2014 (Chapuis et al. 2011). Robertson et al. (2016) proposed a relationship between the area of invasive mammal eradications and their cost from previous eradication projects. According to this work, a mammal population spread over an area of around 1,000 km^2 could be eradicated with few millions US dollars. If the species is not banned from Europe, the possibility of new introductions is high and therefore further management actions will be needed. Control costs will then increase with every new case of an introduced, established and spreading population.
Social and human health impacts			
2.26. How important is social, human health or other impact (not directly included in any earlier categories)	minimal	low	*Callosciurus finalysonii* sampled in pet stores in Italy tested positive for *Dicrocoelium dendriticum* (d'Ovidio

caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).			et al. 2014) that could infect humans (Gualdieri et al. 2011; Jeandron et al. 2011). Several fungal diseases were found in animals culled in Southern Italy: *Cryptococcus neoformans*, *Debaryomyces hansenii*, *Meyerozyma guilliermondii*, *Hanseniaspora thailandica*. The latter species originated from the Indochinese area and was probably introduced in Italy with the squirrels (Iatta et al. 2015). However, no information is available on the human health impact associated. A bornavirus associated with variegated squirrel *Sciurus variegatoides* was reported to have lethal zoonotic effects on three squirrel breeders of the same private squirrel-breeding association in Germany (Hoffman et al. 2015). However, all patients had pre-existing medical conditions and it is unknown whether this virus could also be present/transmitted by *C. finlaysonii*.
2.27. 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.	minimal	low	No information has been found on the issue.
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	minimal	low	Alien tree squirrels have been linked to the introduction of novel parasites and diseases including the spread of zoonotic disease (e.g. Dozières et al. 2010; Bertolino & Lurz 2013; Romero et al., 2014, 2015). As an illustration, introduced populations of the related Pallas's squirrel in Europe were shown to host a number of co-introduced macroparasites such as lice and nematodes but these pathogen guilds were dominated by a few specialist taxa imported with the founders and were considered a minimal sanitary risks for both native fauna and humans in urbanized habitats where the animals were sampled (Dozières et al. 2010). Currently,

			data for *C. finlaysonii* are largely lacking, but the risk of disease transmission, and introduced individuals acting as vectors for parasites and diseases that can harm native wildlife (and potentially humans) should be considered (Lurz 2014). *Callosciurus finalysonii* sampled in pet stores in Italy tested positive for *Dicrocoelium dendriticum* (d'Ovidio et al. 2014) that could infect humans (Gualdieri et al. 2011; Jeandron et al. 2011). Several fungal diseases were found in animals culled in Southern Italy: *Cryptococcus neoformans*, *Debaryomyces hansenii*, *Meyerozyma guilliermondii*, *Hanseniaspora thailandica*. The latter species originated from the Indochinese area and was probably introduced in Italy with the squirrels (Iatta et al. 2015). The species could probably be preyed on by many mammals and raptors, but there are no data available in the literature on the species that could effectively prey on them and possible effects.
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	minimal	low	No other impacts documented.
2.30. 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?	major	medium	Data from the native range are missing. Predators, parasites and pathogens present in Italy did not limit the spread of the species (Aloise & Bertolino 2005; Aloise et al. 2011).

ANNEXES

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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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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 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.

[10] Not to be confused with „no impact“.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.*. 2017)

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[11]	**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.*

[11] 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.

	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** 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*

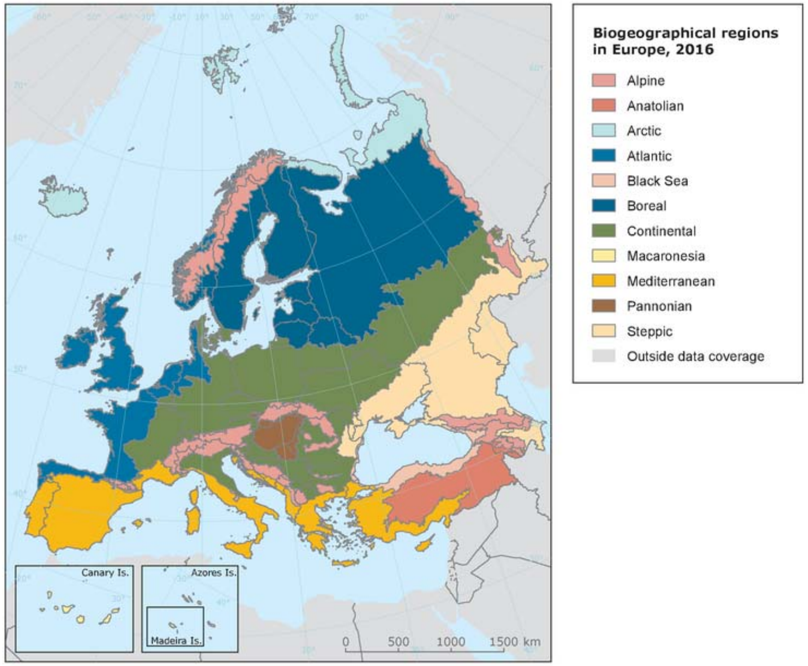
			composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living 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.*

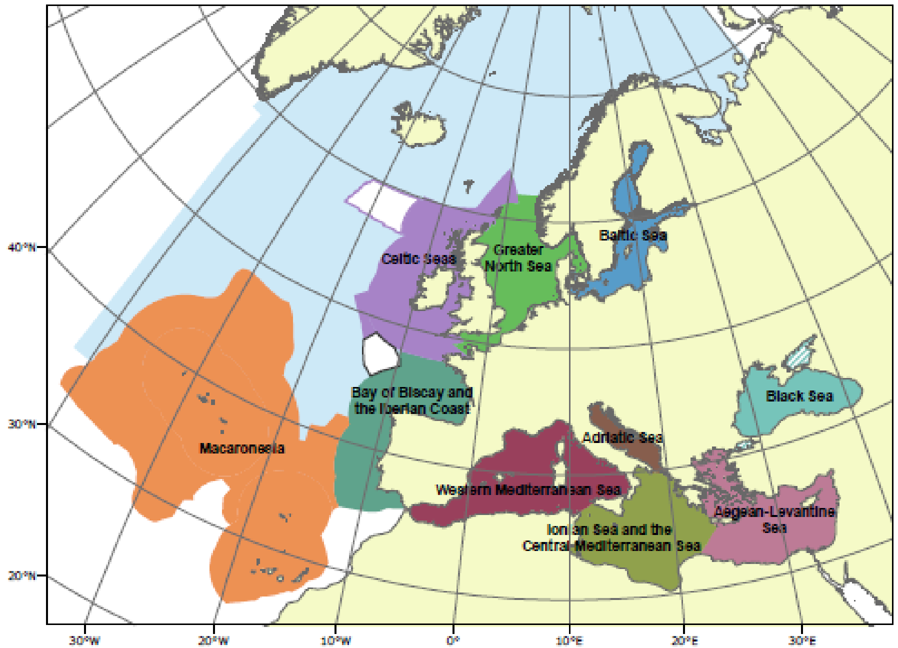
ANNEX V EU Biogeographic Regions and MSFD Subregions

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 - Species Distribution Model

Daniel Chapman - 20th May 2018

Aim

To project the climatic suitability for potential establishment of *Callosciurus finlaysonii* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF), VertNet, iNaturalist, iDigBio, and from a database of Italian occurrences (Sandro Bertolino, *pers. comm.*). We scrutinised occurrence records from regions where the species is not known to be established and removed any that appeared to be dubious 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 (Figure 1a). This resulted in a total of only 58 grid cells containing records of *C. finlaysonii* for the modelling (Figure 1a), which is a very low number for distribution modelling.

Climate data were taken from '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. Consideration of the likely limiting factors on establishment in Europe led to selection of the following climate variables were used in the modelling:

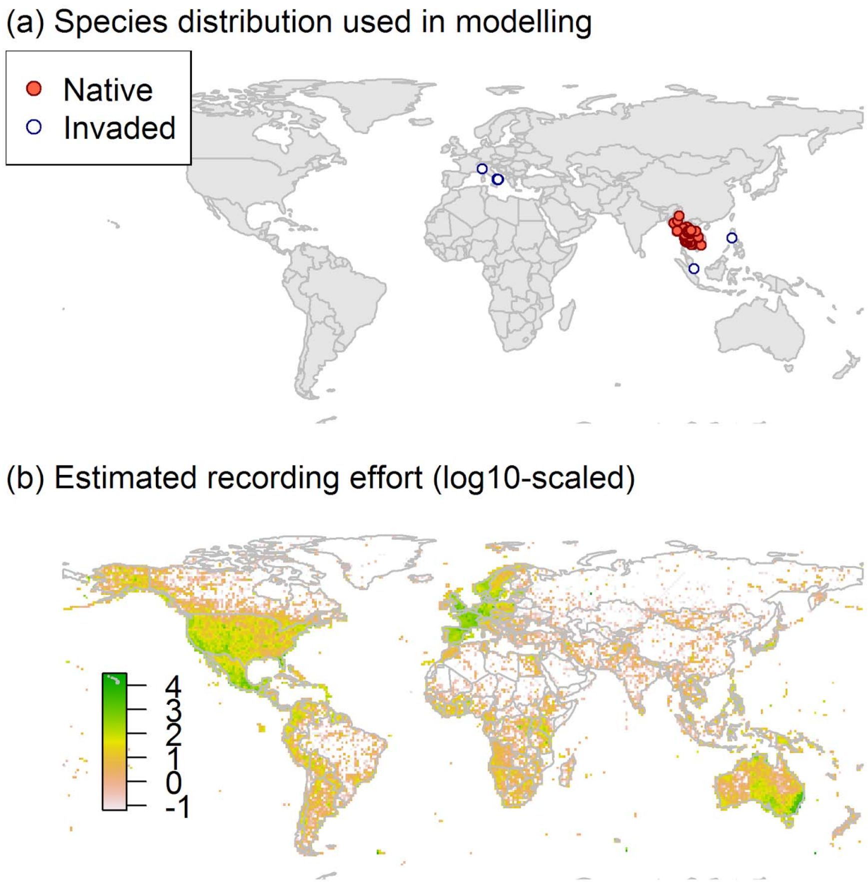
- Minimum temperature of the coldest month (Bio6 °C) reflecting winter cold stress.
- Mean temperature of the warmest quarter (Bio10 °C) reflecting the summer thermal regime.
- Annual precipitation (mm, log+1 transformed) reflecting moisture availability.

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathway (RCP) 4.5 and 8.5 were also obtained. For both scenarios, 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).

RCP 4.5 is a moderate climate change scenario in which CO_2 concentrations increase to approximately 575 ppm by the 2070s and then stabilise, resulting in a modelled global temperature rise of 1.8 C by 2100. RCP8.5 is the most extreme of the RCP scenarios, and may therefore represent the worst case scenario for reasonably anticipated climate change. In RCP8.5 atmospheric CO_2 concentrations increase to approximately 850 ppm by the 2070s, resulting in a modelled global mean temperature rise of 3.7 °C by 2100.

Finally, the recording density of mammals on GBIF was obtained as a proxy for spatial recording effort bias (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Callosciurus finlaysonii* and used in the modelling, showing the native range and (b) a proxy for recording effort – the number of mammal records held by the Global Biodiversity Information Facility, displayed on a $\log_{10}$ scale.



Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7 (Thuiller et al., 2009, 2016). Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale (Elith et al., 2010), we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to. Therefore background samples (pseudo-absences) were sampled from two distinct regions:

- An accessible background includes places close to *C. finlaysonii* populations, in which the species is likely to have had sufficient time to disperse and sample the range of environments. *Callosciurus* species are generally considered to have relatively low dispersal abilities, and the most peripheral southern Italian record was approximately 40 km from other populations. Therefore we defined the accessible background as a 40 km buffer around non-native records, and a 100 km buffer around the minimum convex polygon bounding native records. Sampling was more restrictive from the invaded range to account for stronger dispersal constraint over a shorter residence time.
- An unsuitable background includes places with an expectation of environmental unsuitability, e.g. places too cold or dry. Absence from these regions should be irrespective of dispersal constraints, allowing inclusion of this background in the modelling. No specific ecophysiological information was available to define the unsuitable region, but based on expert opinion that cold and drought are likely to be limits on *C. finlaysonii* occurrence in Europe unsuitability was defined as:
  - Minimum temperature of the coldest month (Bio6) < -1 °C, OR
  - Mean temperature of the warmest quarter (Bio10) < 19 °C, OR
  - Annual precipitation (Bio12) < 600 mm.

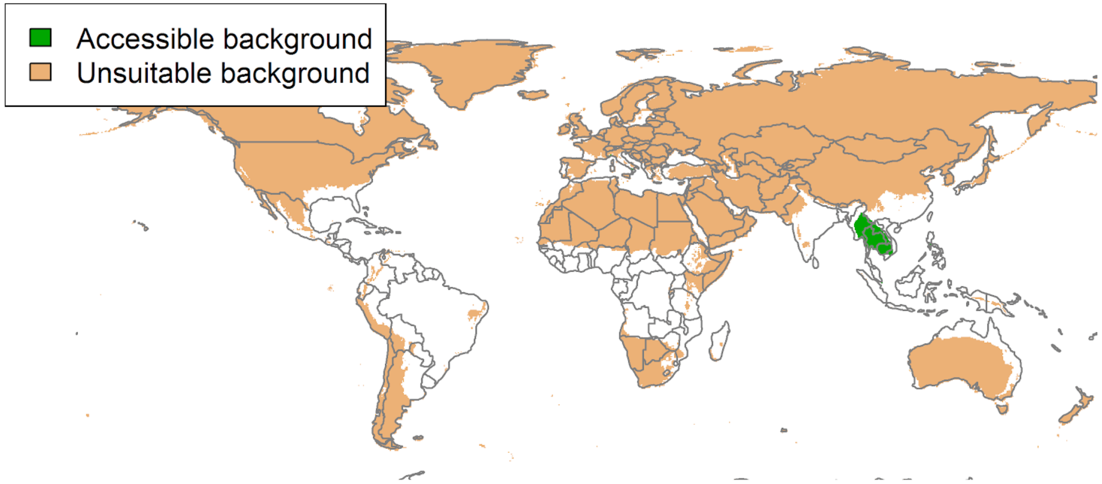
None of the 58 occurrences fell within the unsuitable background.

Ten random background samples were obtained:

- From the accessible background 58 samples were drawn, which is the same number as the occurrences. Sampling was performed with similar recording bias as the distribution data using the target group approach (Phillips, 2009). In this, sampling of background grid cells was weighted in proportion to mammal GBIF recording density (Figure 1b). Taking the same number of background samples as occurrences ensured the background sample had the same level of bias as the data.
- From the unsuitable background 3000 simple random samples were taken. Sampling was not adjusted for recording biases as we are confident of absence from these regions.

Model testing on other datasets has shown that this method is not overly sensitive to the choice of buffer radius for the accessible background or the number of unsuitable background samples.

Figure 2. The background regions from which 'pseudo-absences' were sampled for modelling. The accessible background is assumed to represent the range of environments the species has had chance to sample. The unsuitable background is assumed to be environmentally unsuitable for the species.



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 (except where specified below) and rescaled using logistic regression:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per effect.
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent (Phillips et al., 2008)

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 calculating the Area Under the Receiver-Operator Curve (AUC) for model predictions on the evaluation data, which were reserved from model fitting. AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected pseudo-absence.

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.

Global model projections were made for the current climate and for the two climate change scenarios, avoiding model extrapolation beyond the ranges of the input variables. The optimal threshold for partitioning the ensemble predictions into suitable and unsuitable regions was determined using the 'minimum ROC distance' method. This finds the threshold where the Receiver-Operator Curve (ROC) is closest to its top left corner, i.e. the point where the false positive rate (one minus specificity) is zero and true positive rate (sensitivity) is one.

Limiting factor maps were produced following Elith et al. (2010). 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. Partial response plots were also produced by predicting suitability across the range of each predictor, with other variables held at near-optimal values.

Results

The ensemble model suggested that at the global scale and resolution of the model suitability for *C. finlaysonii* was most strongly determined by precipitation, with strong effects of both temperature variables (Table 1). However, for the temperature predictors, there was substantial variation in the partial response plots between algorithms (Figure 3), highlighting the value in reducing this uncertainty through the use of ensemble model (Table 1).

Global projection of the ensemble model in current climatic conditions indicates that the native and known invaded records in Europe and Asia generally fell within regions predicted to have high suitability (Figure 4). In the native range, the model suggested a wider region of potentially suitable climate than is currently occupied. To some extent this is supported by the introduced populations in Singapore and the Philippines, but it does suggest that the current native range in continental Asia may be limited by factors other than climate, such as habitat availability of biotic interactions.

In Europe, the clusters of occurrences in northern and southern Italy were projected as being in climatically suitable locations (Figure 5). Beyond these, the model suggests climatically suitable regions occur widely around the eastern Mediterranean coast. The model also predicts suitable regions in the southern tip of Spain and in Portugal. Establishment in northern and eastern Europe was predicted to be prevented by low winter temperatures, while low summer temperatures were suggested as being more limiting in the Atlantic region (Figure 6). In unsuitable parts of the Mediterranean, low precipitation was identified as a strong potential limiting factor. Assuming thermal conditions were suitable in those regions, it remains possible that the species could establish where it has access to permanent water sources.

Predictions of the model for the 2070s, under the moderate RCP4.5 and extreme RCP8.5 climate change scenarios, suggest an eastwards shift in suitability in the Mediterranean (Figure 7-8). Suitable regions in Iberia appear to become too dry for the species, while warming benefits the species along the Adriatic coast. Climatically suitable conditions also appear in Western Europe, for example in the Atlantic coasts of Portugal, Spain and France and even as far north as Belgium and Netherlands. This is presumably driven by a combinations of warmer summers and milder winters.

In terms of Biogeographical Regions (Bundesamt fur Naturschutz (BfN), 2003), the Mediterranean and Black Sea regions are predicted most suitable for invasion in the current climate (Figure 9). Under the future climate scenarios, suitability in the Atlantic region increases markedly.

Table 1. Summary of the cross-validation predictive performance (AUC) and variable importances of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to ten different background samples of the data.

Algorithm	**AUC**	**In the ensemble**	**Variable importance**		
			Minimum temperature of coldest month	**Mean temperature of warmest quarter**	**Annual precipitation**
GAM	0.9911	yes	31%	26%	43%
Maxent	0.9899	yes	40%	14%	46%
GLM	0.9869	yes	38%	21%	42%
ANN	0.9866	yes	35%	30%	35%
MARS	0.9860	yes	35%	24%	40%
RF	0.9849	yes	15%	25%	61%
GBM	0.9844	yes	34%	22%	43%
Ensemble	**0.9922**		**33%**	**23%**	**44%**

Figure 3. Partial response plots from the fitted models, ordered from most to least important. 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.

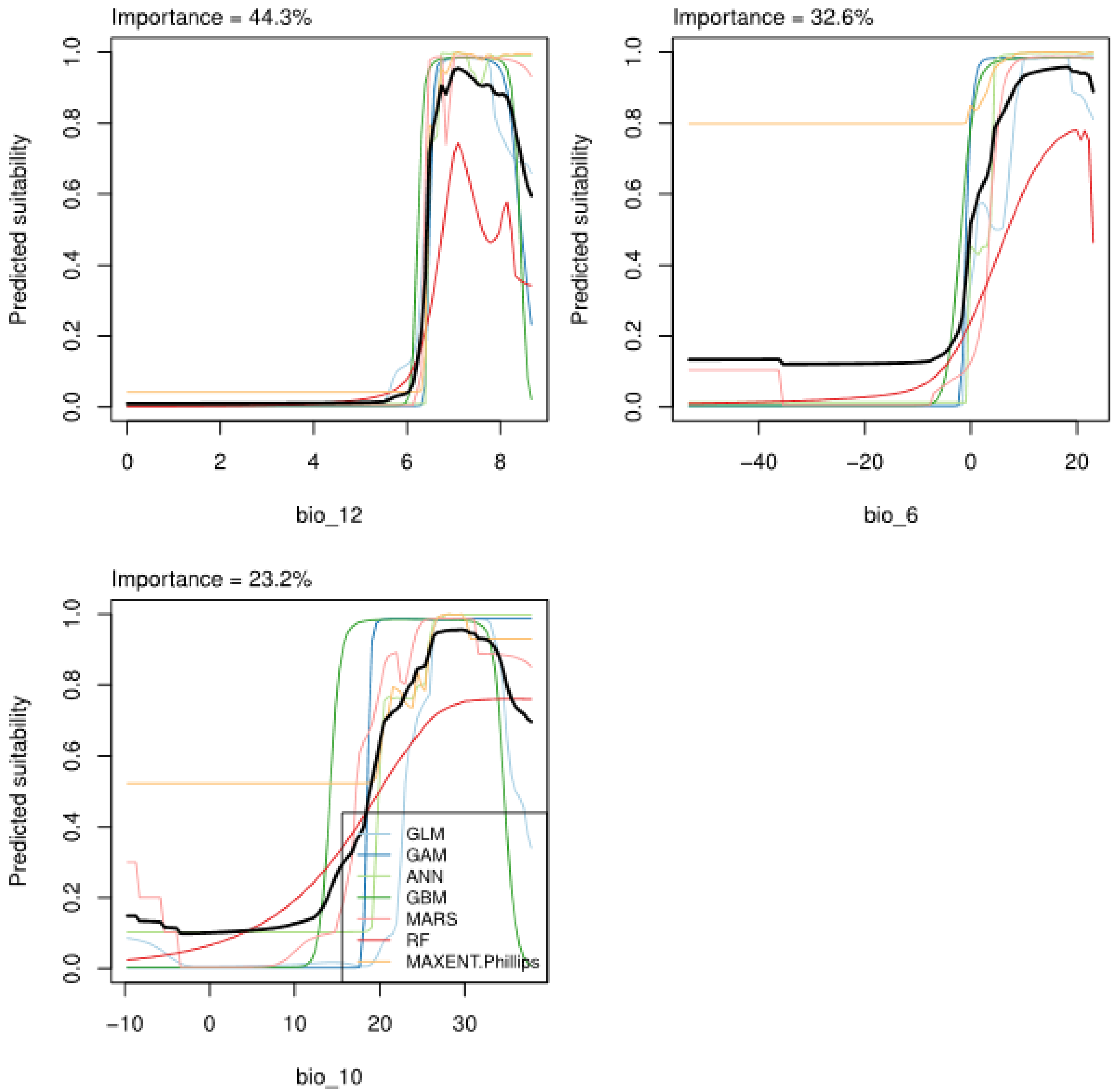

Importance = 44.3%
Predicted suitability
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
bio_12
Importance = 32.6%
Predicted suitability
0.0
0.2
0.4
0.6
0.8
1.0
−40
−20
0
20
bio_6
Importance = 23.2%
Predicted suitability
0.0
0.2
0.4
0.6
0.8
1.0
−10
0
10
20
30
bio_10
GLM
GAM
ANN
GBM
MARS
RF
MAXENT.Phillips

Figure 4. (a) Projected global suitability for *Callosciurus finlaysonii* 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. Red shading indicates suitability. White areas have climatic conditions outside the range of the training data so were excluded from the projection. (b) Uncertainty in the suitability projections, expressed as the standard deviation of projections from different algorithms in the ensemble model.

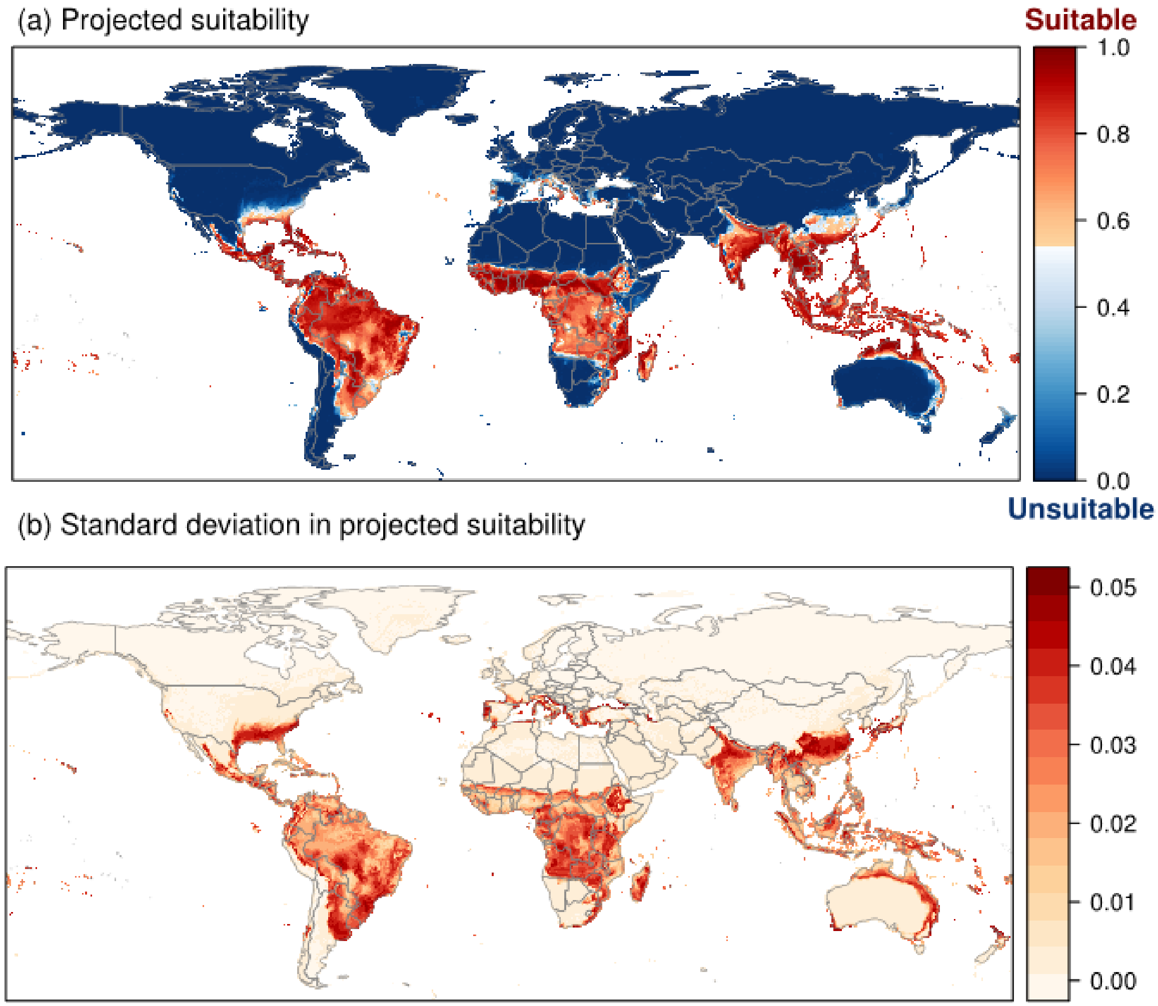


Figure 5. Projected current suitability for *Callosciurus finlaysonii* establishment in Europe and the Mediterranean region. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

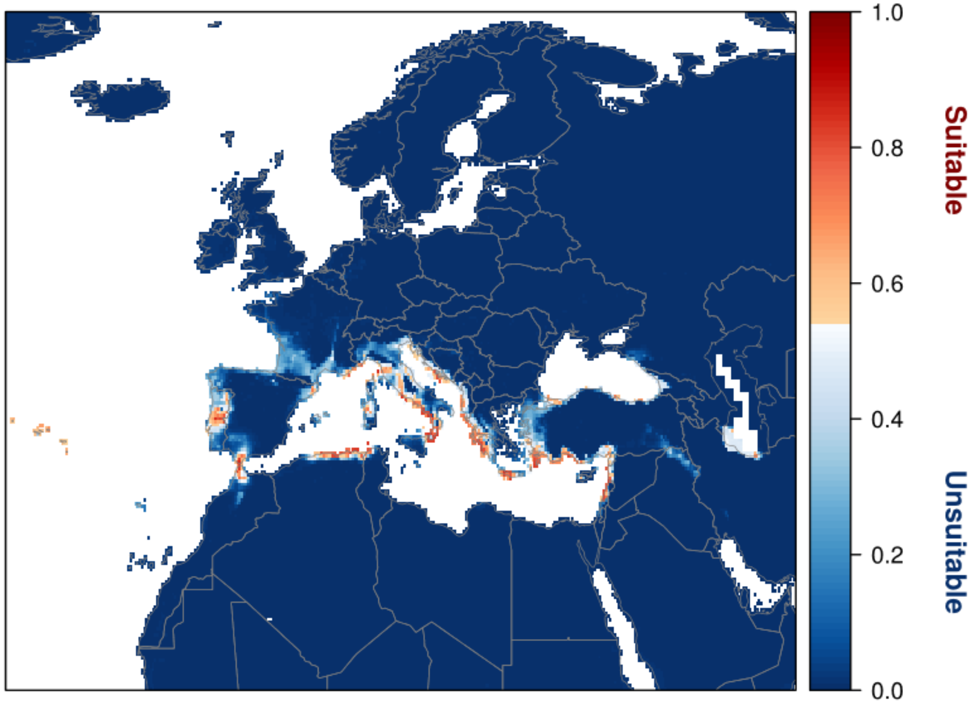


Figure 6. Limiting factor map for *Callosciurus finlaysonii* establishment in Europe and the Mediterranean region in the current climate. Shading shows the predictor variable most strongly limiting projected suitability.

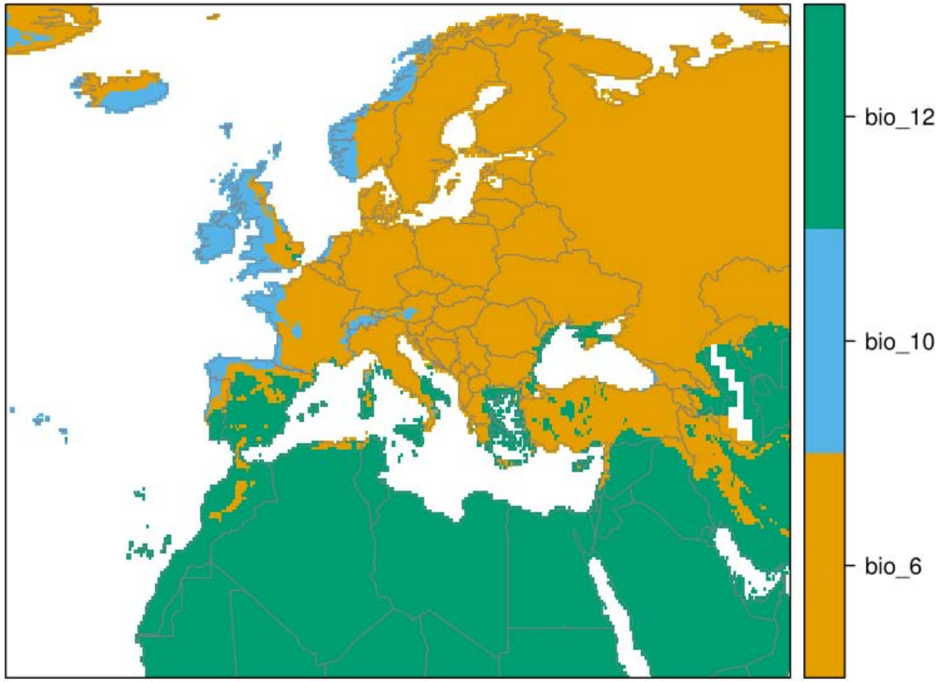


Figure 7. Projected suitability for *Callosciurus finlaysonii* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5.

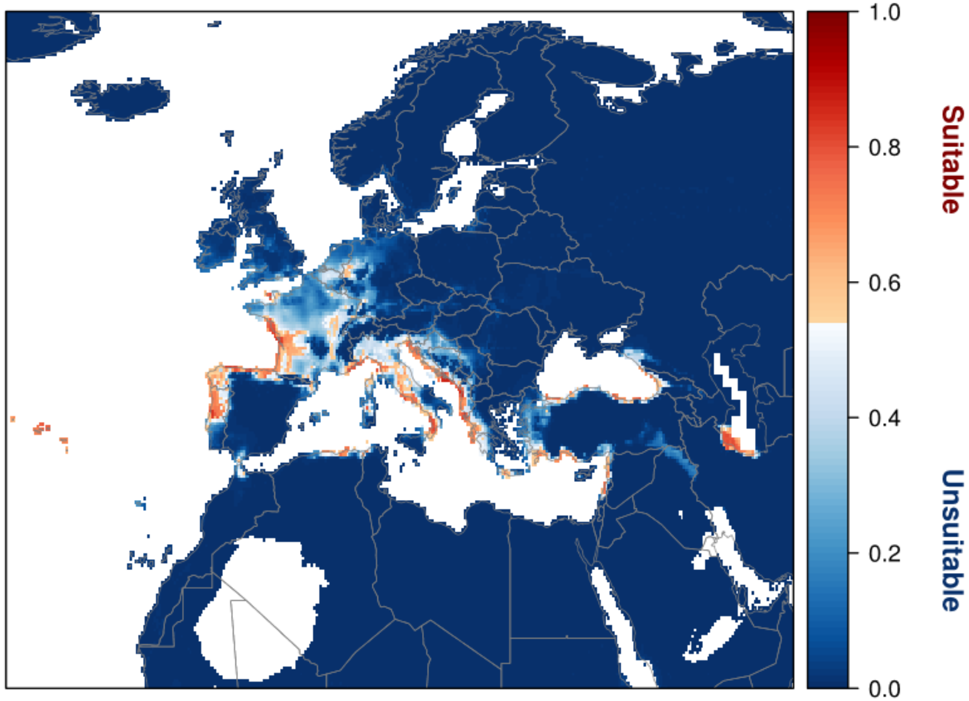


Figure 8. Projected suitability for *Callosciurus finlaysonii* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP8.5, equivalent to Figure 5.

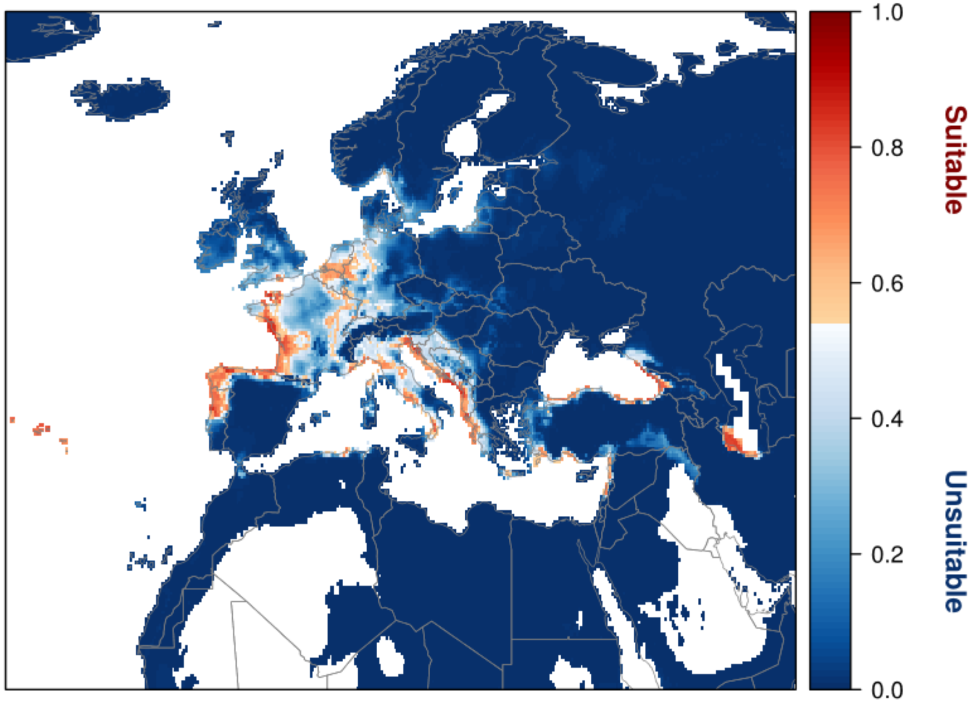
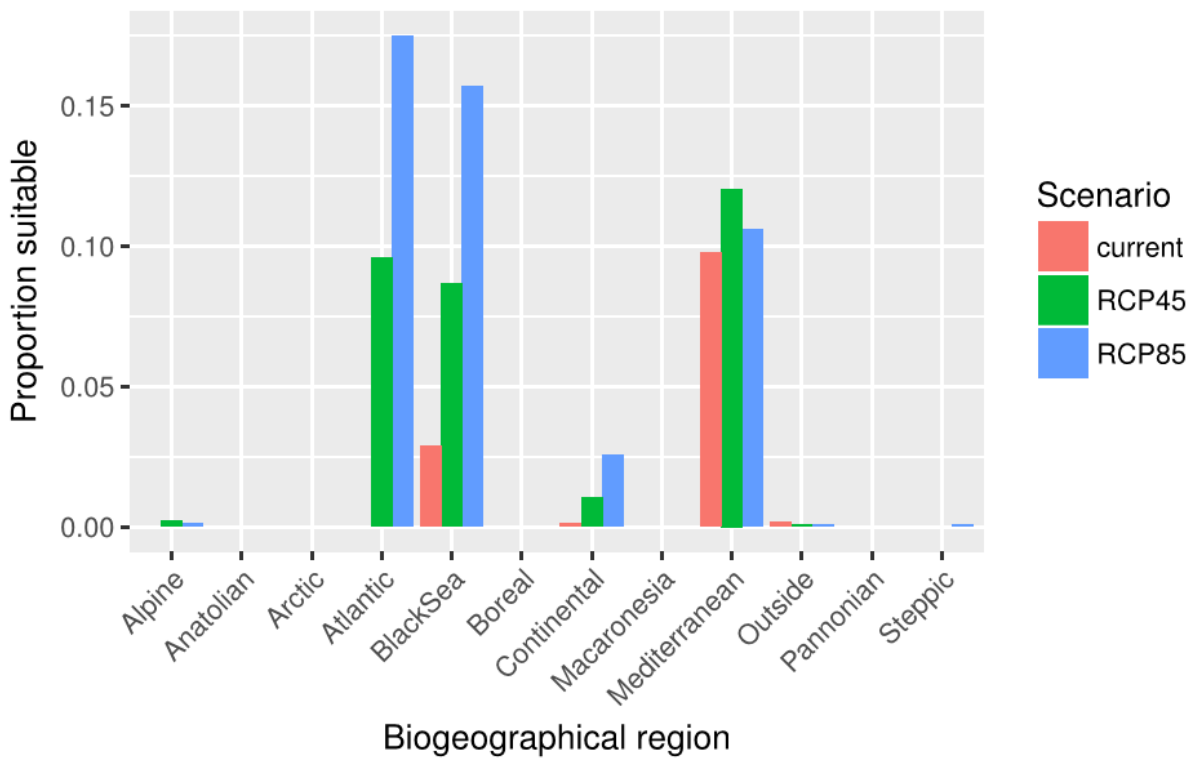
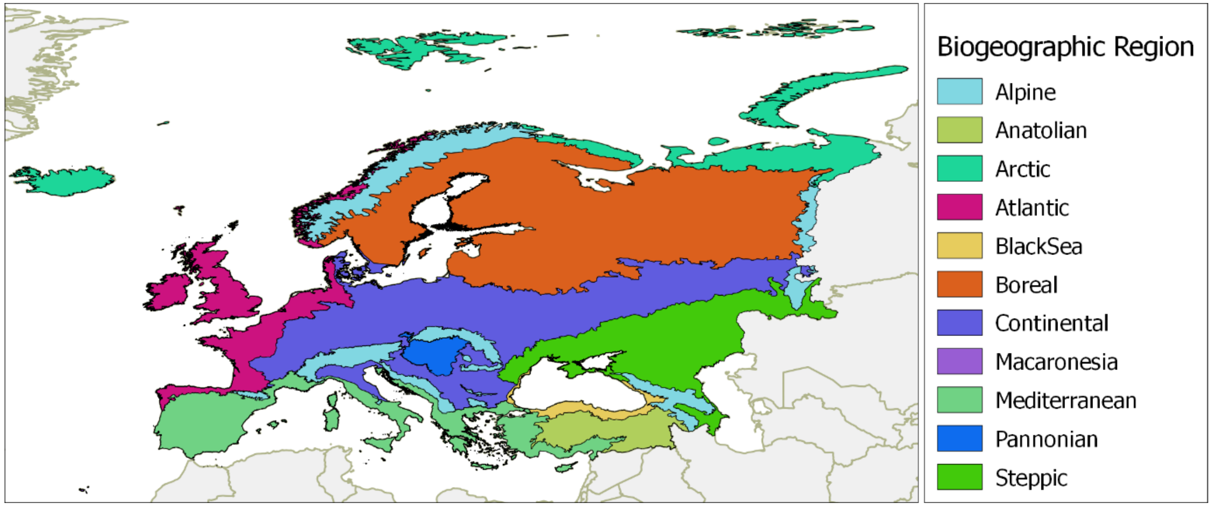


Figure 8. Variation in projected suitability among Biogeographical regions of Europe (Bundesamt fur Naturschutz (BfN), 2003). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under emissions scenarios RCP4.5 and RCP8.5. The coverage of each region is shown in the map below.


Proportion suitable
0.15
0.10
0.05
0.00
Alpine
Anatolian
Arctic
Atlantic
BlackSea
Boreal
Continental
Macaronesia
Mediterranean
Outside
Pannonian
Steppic
Biogeographical region
Scenario
current
RCP45
RCP85



Caveats to the modelling

Modelling the potential distributions of range-expanding species is always difficult and uncertain.

The modelling here is subject to a high degree of uncertainty for the following reasons:

- An unusually small number of distribution records was available for the modelling, possibly not capturing the full range of conditions in which the species can establish.
- There was no ecophysiological information available to contribute to definition of the unsuitable background region.
- *Callosciurus* species are known to be adaptable and may be able to expand their niche into cooler conditions than are currently observed.
- The role of precipitation as a limiting factor in Iberia and other parts of the Mediterranean may be overstated if the species has access to permanent water sources.

The model did not include other variables potentially affecting occurrence of the species, including habitat availability or biotic interactions. These were not included because of the very small number of distribution records.

To remove spatial recording biases, the selection of the background sample was weighted by the density of mammal records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, a number of factors mean this may not be the perfect null model for species recording, especially because additional data sources to GBIF were used.

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Template for Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	*Callosciurus finlaysonii*
Species (common name)	Finlayson's Squirrel
Author(s)	PA Robertson
Date Completed	25 October 2018
Reviewers	Tim Adriaens, Sandro Bertolino, Craig Shuttleworth

Summary
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.

Methods to achieve prevention
This species is kept and traded through the pet trade. It has been introduced into Italy through the accidental or deliberate release of captive animals. The adoption and enforcement of appropriate legislation (Art. 7 of the Regulation (EU) 1143/2014) and codes of best practice targeted to commercial and non-commercial owners in Europe to reduce the risks posed by these pathways should reduce the probability of further introductions. Raising awareness of the problems posed by the release or presence of this species, and invasive species in general, should reduce the risk of further escapes and the rapid reporting of new populations to support a rapid response.

Methods to achieve eradication
Live-trapping is already widely used to control invasive alien squirrels and is likely to be the most effective method for this species. More experience is needed to determine the most cost-effective designs and deployment of traps for this species, but experience from the control of grey and Pallas' squirrel provides guidance on suitable approaches. Shooting can be an effective tool to supplement trapping but its use can be limited by social and local regulatory considerations. The effectiveness of shooting can be enhanced by its use alongside baiting and nest disturbance. A range of other possible control approaches are described, including fertility control, the use of toxins and biological control by native predators. These are considered unlikely to contribute to the control of this species without significant further development, legislative changes or evidence of their importance. Suitable surveillance methods include the use of visual searches, hair tubes, camera traps and the use of baited feeding sites.

Methods to achieve management
A combination of trapping and shooting are also likely to provide the most cost-effective methods for the long-term management of this species. The objectives of long-term management are likely to include limiting commercial damage to trees through bark stripping and reducing impacts on native species such as native tree squirrels.

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	**Managing pathways** Finlayson's squirrel is held within the pet trade, and accidental and deliberate releases of pet animals are thought to be the pathway of introduction to Europe. The adoption and enforcement of appropriate legislation (Art. 7 of the Regulation (EU) 1143/2014) and codes of best practice targeted to commercial and non-commercial owners in Europe to reduce the risks posed by these pathways should reduce the probability of further introductions (Bertolino 2009).		
	Effective reporting of new incursions Finlayson's squirrel are a novel species for Europe, given their variegated coat colour they are readily distinguished from native Calabrian black squirrel, but could be confused by non-experts with the Eurasian red squirrel.. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the species can become established.		
	Raising awareness Raising public awareness of the risks posed by invasive alien species in general, and ornamental squirrels in particular, with examples on the impacts. This can include the production of targeted publicity and identification material, and the involvement of the general public, along with key stakeholders, in citizen science initiatives.		
Methods to achieve eradication	**Trapping** A wide variety of trap designs have been developed and used for the control and research of similar squirrel species. Trapping is the main method used to remove the invasive grey squirrel from large islands in the UK and Pallas' Squirrel from Flanders (Schuchert et al 2014, Adriaens et al 2015). Traps for squirrels include both lethal and cage designs. The selection of which type of trap to deploy is determined by a number of factors. Cage traps are less likely to raise welfare concerns, and live capture allows any non-target captures to be released. However, traps must be	Finalsyson's squirrels have been removed with live-traps from a small area in Southern Italy (Ricciardi et al 2013), but no details of the effects on the population are available. Traps have been widely used to eradicate and control other species of invasive tree squirrel, including grey squirrel and Pallas' Squirrel. The costs of trapping for eradication are largely determined by the area over which the species has spread (Robertson et al 2017) rather than the	**High** – There is a large existing literature and practical experience of using traps to control similar species in Europe

	checked frequently, at least daily and in many cases more frequently, to avoid animals remaining in the trap for an extended period and risks of their injury (Shuttleworth et al. 2018). This need for regular checking increases the man-power requirements and hence the cost of use. Lethal traps, including a range of spring trap designs, can also be effective and may be cheaper to operate. However, they pose a higher risk to individual non-target species, for example if there are protected native squirrels in the area. This is the case for grey squirrel trapping in areas with remaining red squirrels in GB, where the use of lethal traps is prohibited to avoid killing the native species. Lethal traps also require more training to be used safely and effectively, poorly set traps risk serious injury and welfare concerns if the animals are not killed cleanly. Traps for squirrels are widely available for sale on the internet, and a variety of designs are freely available for their construction (Search term 'Squirrel Traps'). No information is currently available on the most effective trap design for use on this species. The general guidance on the availability and use of traps for grey squirrels provides a useful background applicable for this species (Mayle et al 2007 although this is currently being updated), including issues such as different trap designs, welfare and non-target considerations, baits and pre-baiting. However, the grey squirrel is considerably larger and spends more time on the forest floor than the smaller Finlayson's squirrel (Gurnell 1987) which is more arboreal (Bertolino et al 2004). As a consequence, the published grey squirrel designs and guidance may need to be refined to be appropriate for Finlayson's squirrel. The native red squirrel is only slightly larger than Finlayson's squirrel and is also largely arboreal. Designs of traps suitable for the capture of red squirrels, and guidance for their setting may be more appropriate. Given the arboreal nature of this species, setting taps on horizontal branches or small platforms may increase their success. Finlayson's squirrel eats buds, fruits, berries as well as insects (Bertolino et al 2004). It is likely that peanut butter, grains such as	number of individual animals. Responding rapidly before the species has dispersed from the initial point of introduction is recommended. Grey squirrels have been completely removed using traps from the 710km^{2} island of Anglesey in GB (Schuchert et al 2014, Shuttleworth et al 2015). This programme required 30 man-years of effort in the period 1998-2013. Pallas's squirrel was eradicated from an area in Flanders, Belgium using traps. Sightings of this species had occurred over an area of nearly 3km^{2} and 250 animals were removed within 5-years at a cost of Euro 200k including surveillance and post-eradication monitoring (Adriaens et al. 2015). A Pallas's squirrel invasion in The Netherlands (Weert) was tackled by rapid eradication at a cost of 330k to run the programme and remove 250 squirrels, but with the help of volunteers (pers. comm. M. La Haye; Dijkstra & Bekker 2012; Dijkstra 2013a,b). Cost are higher for established populations with a higher number of animals, such as in France where control actions were planned at abut 100k per annum for the period 2011-2014 (Chapuis et al. 2011). All traps carry a number of risks to the welfare of the captured animal and to non-target species that may also be caught (Shuttleworth et al. 2018). In general terms, well designed and operated cage traps are likely to carry fewer welfare risks compared to lethal designs and	

	maize, fruit and vegetables will all provide suitable baits. Baiting but not setting the trap for a few days may ensure the target has more confidence to enter once the trap is set. Recent developments have also produced self-resetting designs of lethal trap using compressed CO2 gas to re-set the trap once tripped, reducing the need to visit traps to reset after each.capture. .https://www.goodnature.co.nz/products/shop/ These traps do not currently have specific approval for use on Finlayson's squirrel	allow any non-target species captured to be released. The use of traps for this species in Europe would need to take into account the legal situation and approval mechanism in the Member State concerned. It is likely that these will restrict the use of a number of traps in certain countries. Member states also vary in their requirements for the frequency of checking traps. Guidance should be sought from national sources before using traps in individual European Member States	
	Shooting Use of lethal firearms by competent marksmen.	Shooting can be an effective method to remove squirrels. It is highly selective and, if used by experienced personnel, provides a humane method of dispatch. However, it is labour intensive and therefore expensive, although it can be an effective technique to use alongside other methods such as trapping. If the objective is eradication of a population, then shooting alone is unlikely to be effective. Finlayson's squirrels make nests from plant material (Bertolino et al 2004; Brown et al., 2012) that can be identified in the tree canopy. A common method to manage the grey squirrel, which also makes nests or 'dreys', is to poke them with long sticks and to shoot animals as they emerge (BASC). There are no records of this method being used to control Finlayson's squirrel. It is reported that Finlayson's squirrel will take food provided by the public (Bertolino et al	**High**

		2004). In GB, grey squirrel control is often conducted at bait stations set in suitable locations for shooting from a camouflaged hide (BASC). The use of different firearms is heavily regulated and the details vary between European 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 and regulations must be consulted before their use. The use of firearms brings risks to health and safety which need to be managed. Their 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.	
	Poisons Toxins have been used to control squirrel population and reduce damage. Warfarin was widely used to control Grey Squirrels after their introduction to the UK (Pepper 1990). However, the EU license for the production and sale of warfarin as a grey squirrel bait ended on 30 September 2014. Manufacturers and stockists are no longer able to sell warfarin to control grey squirrels.	No toxin has been approved for use on Finlayson's squirrel, and with the removal of Warfarin, no toxins are licensed for use against squirrels in Europe	**High** – When they were legal, toxins were widely used for squirrel control.
	Biological Control Predation by native predators is a possible limiting factor for invasive alien species in some circumstances. Where these native species have been supressed for other reasons, such as historic persecution, then their recovery may help limit the populations of invasive species. There is evidence that the recovery of native pine marten populations in Ireland and GB has been associated with significant declines in the	This possible control mechanism has attracted significant public and scientific interest in GB for the potential control of the invasive grey squirrel. However, the strength of this effect is still uncertain, and other factors are likely to influence pine marten populations in many areas, limiting the scope.	**Low** – This mechanism is still under investigation as a practical tool for the grey squirrel in GB. There is no evidence to suggest

	invasive grey squirrel, while populations of the native red squirrel which has co-evolved with this predator have recovered (Sheehy et al 2018). There is no evidence to suggest such an effect on Finlayson's squirrel.	There is no evidence to suggest such an effect would also apply to Finlayson's squirrel, and the more arboreal nature of this species may reduce the scope for such an effect.	a similar effect is relevant to Finlayson's squirrel
	Fertility Control A variety of fertility control methods have been tested for use on this species under experimental conditions. These have included the use of slow-release implants containing the gonadotrophin-releasing hormone (GnRH) agonist deslorelin, ovarian toxins (Burd 2014), and the use of immunocontraceptive vaccines targeting egg surface proteins (Duckworth et al. 2007) or GnRH (Miller et al. 2008). Methods based on the injection of captive animals have been licensed for use on other mammalian species. https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/programs/nwrc/research-areas/SA_Reproductive_Control/CT_Gonacon http://www.pzpinfo.org/pzp.html A variety of orally delivered products are currently under development but not currently licensed for use. More widely, the use of these fertility control methods has been restricted to captive or experimental conditions. There are currently no examples where these methods have been used to achieve wide-scale vertebrate population control or eradication.	Fertility control has not currently been used to achieve the wide-scale control of free-living wildlife populations. While a variety of methods are available or under development, there remain significant barriers to their effective wide-scale use. These include cost, relative effectiveness compared to lethal control, the development of safe and selective delivery systems, the ability of populations to compensate through increased reproductive output of untreated animals, and the product licensing requirements. While work is underway on all of these issues, further development is needed before they can be effectively used on a significant scale (IUCN 2017). However, surgical sterilization has been applied for squirrel control in urban areas in Italy. Although they come at a relatively high price (100-130€ per animal for the surgery only) and the method has some practical limitations, it can provide an alternative to lethal control of small populations in urban areas or small areas where sensitivity to animal welfare is high. In terms of the time taken to achieve the intended reduction in size of a target wildlife population, culling will always be more efficient than fertility control because reducing population recruitment cannot generate a more rapid population decline than the natural	**Medium** – These methods are currently under development and the literature and experience of their wide-scale use remains limited.

		mortality rate allows (McLeod and Saunders, 2014). This is likely to limit the use of this method for eradication as culling will typically provide a cheaper and more rapid response. Nevertheless. fertility control offers an attractive approach to wildlife management given the increasing concerns about environmental and welfare impacts of lethal management techniques and the increasing constraints on their use (e.g. Fagerstone et al. 2010). In principle, fertility control generally meets with relatively greater public acceptance than lethal control (e.g. Barr et al. 2002), although the approach is not without welfare and ethical issues (Hampton et al. 2015). When lethal control is considered unacceptable or unfeasible, for instance for iconic species or in peri-urban environments, fertility control once available may be a useful option for managing overabundant wildlife populations.	
	Surveillance Achieving eradication requires methods to identify the presence of a species in an area and to assess changes in abundance as the species is controlled. Trapping based control programmes typically use the change in the number of animals trapped per unit effort to assess changes in abundance, although this can be sensitive to the proportion of trap-shy individuals in the population. Independent surveillance methods to determine changes in abundance of animals as control proceeds, or to assess their presence or absence in an area are often required. Gurnell et al (2001) describe a range of practical techniques for surveying and monitoring squirrels. Visual	Ancillotto et al (2018) conducted visual surveys and hair tube sampling in a peri-urban landscape of southern Italy to compare the effectiveness of these two methods in assessing presence of Finlayson's squirrel. Both visual and hair tube sampling effectively assessed the species' presence, but hair tubes resulted in fewer false absences. Moreover, when controlling for the costs of labour and equipment, hair tubes were 33.1% less expensive than visual sampling.	**High** – Surveillance methods developed for other tree squirrels are likely to be applicable to this species

	surveys or hair tubes set in trees have already been used successfully for Finlayson's squirrel (Ancillotto et al 2018). Camera traps can be an effective method of monitoring (O'Connell et al 2010) and were used alongside the control of Pallas' squirrel in. Flanders (Adriaens et al 2015). If Finlayson's squirrels come regularly to baited food stations then these can provide a useful focus for visual or camera based surveillance. Citizen science may also contribute to both monitoring and surveillance.		
Methods to achieve management	All of the methods described to support eradication can also be used to manage existing Finlayson's squirrel populations.	See above.	See above.
	Limiting commercial damage to trees through bark stripping Finlayson's squirrel is reported to strip the bark from trees, a behaviour common to a number of tree squirrels and one that can cause significant economic damage. In GB, the management of grey squirrel populations in commercial woodlands aims to reduce levels of tree damage to economically acceptable levels. This includes the identification of forest stands most likely to suffer economic damage, and the use of a combination of trapping and shooting on a periodic basis to suppress squirrel densities. Mayle et al (2007) provide guidelines for the control of grey squirrel damage to woodlands, although these are currently being updated.	See above.	See above.
	Reducing impacts on native species Invasive alien tree squirrels can compete with native species through predation, competition for resources and disease transmission (see section of impacts) In particular, they can compete with native squirrels, through competition for resources and by the transmission of disease (Rushton et al 2000. Gurnell et al. 2004; Mazzamuto et al. 2017a,b). Both of these mechanisms are involved in the decline of the native red squirrel in GB and Ireland in the face of the spreading invasive grey squirrel, but not in Italy where the squirrelox virus was not detected (Romeo et al. 2018).	See above.	See above.

	Reducing the density of invasive squirrels, and limiting their spread into new areas, can be the objectives of programmes to reduce their impacts on native species. Pepper and Patterson (1998) and Shuttleworth et al (2016) discuss and provide guidelines for the conservation of native tree squirrels in the face of competition from invasive species, based on the red and grey squirrel in GB.		

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2[1]

Name of organism: *Xenopus laevis* (Daudin, 1802)

Author(s) 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: John Measey, Centre for Invasion Biology, Department of Botany & Zoology, Stellenbosch University, Stellenbosch, South Africa
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This risk assessment has been peer-reviewed by two independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study.

Date of completion: 25/10/2018

[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

Contents

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**[2]	**COMMENT**
Summarise Entry[3]	very likely	high	The species is already present in the risk assessment area thanks to two main pathways (pet trade and research) which are still active. Facilities hosting captive populations are already present in several countries, hence the risk of entry is very high.
Summarise Establishment[4]	very likely	high	The species is already successfully established in the risk assessment area. There are also evidences that suitable conditions for the species are present in other countries where there are not yet established populations.
Summarise Spread[5]	rapidly	medium	Many studies have shown that the species may rapidly disperse by natural means (i.e. through ecological corridors) in the risk assessment area, thanks to both the naturally occurring environmental conditions and the species' intrinsic ecological and biological features.
Summarise Impact[6]	moderate	medium	There is evidence of *Xenopus laevis* potentially functioning as a reservoir for *Bd* and other pathogens. However, to date there is no evidence of this or that *Xenopus laevis* has caused impact on native amphibians through this mechanism. Other direct impacts of this species are likely to be more severe. For example, native species which compete with *Xenopus laevis*, or which are included on its diet (particularly the macro-invertebrate communities), may also be affected.

[2] In a scale of low / medium / high, see Annex III

[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly

[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

Conclusion of the risk assessment[7]	moderate	medium	The species is known to be invasive in the risk assessment area, and there is evidence that further releases (or spread) may occur in areas which are not yet colonised, leading to the successful establishment of other populations, hence increasing the overall impact associated with the occurrence of *Xenopus laevis* in the wild.

[7] In a scale of low / moderate / high

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Austria				
Belgium	Yes		Yes	
Bulgaria				
Croatia				
Cyprus				
Czech Republic				
Denmark			Yes	
Estonia				
Finland				
France	Yes	Yes	Yes	Yes
Germany	Yes		Yes	
Greece			Yes	
Hungary				
Ireland			Yes	
Italy	Yes	Yes	Yes	Yes
Latvia				
Lithuania				
Luxembourg				
Malta				
Netherlands	Yes		Yes	
Poland				
Portugal	Yes	Yes	Yes	Yes
Romania				

Slovakia				
Slovenia				
Spain	Yes		Yes	
Sweden	Yes			
United Kingdom	Yes	?	Yes	

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Alpine			Yes	
Atlantic	Yes	Yes	Yes	Yes
Black Sea				
Boreal				
Continental			Yes	
Mediterranean	Yes	Yes	Yes	Yes
Pannonian				
Steppic				

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Established (future)	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				

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	This risk assessment covers only one species, *Xenopus laevis* (Daudin, 1802), the African Clawed Frog or Common Platanna (Class: Amphibia; Order: Anura; Family: Pipidae; Genus: *Xenopus*). The African Clawed Frog is also known as Platanna, Common Platanna, Common Clawed Frog, Clawed Toad, Clawed Frog, Upland Clawed Frog, Smooth Clawed Frog, African Clawed Toad, Upland Clawed Frog, Common Clawed Frog, Common Clawed Toad, African Clawed Frog (Frost 2018). *X. laevis* belongs to a genus that comprises at least 29 species, half of which occur in Central Africa (Evans et al. 2015). According to Evans et al. (2015) although *Xenopus* is easily distinguished from other frog genera, discriminating the relevant species based solely on morphological characters can be difficult. Distinguishing *X. laevis* from other species of the same genus is usually no problem where they occur, as no other species is as large as *X. laevis*. Otherwise, some difficulties may be faced in the case of hybrids (John Measey pers. comm. 2018). As summarised by Measey (2016) *X. laevis* has undergone significant taxonomic revision following a comprehensive molecular study by Furman et al. (2015). The result of this revision is that what was previously known as *X. l. laevis* is now known as *X. laevis* with all other subspecies being recognised as full species, and some newly described species included as well (Evans et al., 2015). In fact, as reported by Furman et al. (2015) within *X. laevis* sensu lato, the analyses show at least four lineages: *X. laevis* (southern Africa, including Malawi and South Africa), *X. poweri* (Central Africa, including Nigeria, Cameroon, Zambia, and Botswana), *X. petersii* (West Central Africa, including the Republic of Congo, western DRC, and Angola) and *X. victorianus* (East Africa, including Kenya, Uganda, Rwanda, Burundi, eastern DRC, and Tanzania). The data potentially support the transfer of *X. l. sudanensis* to the synonymy of *X. poweri* (instead of *X. laevis*), while *X. l. bunyoniensis* should be tentatively considered a synonym of *X. victorianus*.

	Reciprocal crosses between individuals of *X. laevis* sensu lato (that were probably from South Africa), and individuals from Uganda or Botswana, both produced fertile offspring, thus gene flow between these species is possible (Furman et al. 2015). *X. laevis* is also known to hybridise with *Xenopus gilli*. The hybrids of these species pose no intrinsic invasive threat, except for the conservation of the latter species, which is also affected by predation and competition by *X. laevis* (Measey et al. 2017). Hybrids are also known in the wild, in hybrid zones, with *X. poweri* (conjecture) and *X. muelleri* (Fischer et al 2000). However, since we cannot exclude the possibility that hybrids are present in trade and/or in the populations established in the wild, this risk assessment should apply to all *X. laevis* hybrids as well. This is justified by the fact that while some physiological features may be different, the overall impact would be the same. As a remark, this assessment is for *X. laevis*, but unless otherwise stated, all statements apply to any hybrids as well as albino *X. laevis* (albino individuals belong exactly to the same X. laevis species). Here follows a list of the most common synonym names of *X. laevis* according to Frost (2018): • *Bufo laevis* Daudin, 1802 • *Dactylethera boiei* (Wagler, 1827) • *Dactylethra bufonia* (Merrem, 1820) • *Dactylethra capensis* Cuvier, 1830 • *Dactylethra delalandii* Cuvier, 1849 • *Dactylethra laevis* (Daudin, 1802) • *Engystoma laevis* (Daudin, 1802) • *Leptopus boiei* (Wagler, 1827) • *Leptopus oxydactylus* Mayer, 1835 • *Pipa africana* Mayer, 1835 • *Pipa bufonia* Merrem, 1820 • *Pipa laevis* (Daudin, 1802) • *Tremeropugus typicus* Smith, 1831 • *Xenopus boiei* Wagler, 1827 • *X. laevis* ssp. *bunyoniensis* Loveridge, 1932 • *X. laevis* ssp. *sudanensis* Perret, 1966

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 wild, in confinement or associated with a pathway of introduction]	*X. laevis* is not difficult to distinguish from other anurans occurring in Europe (either native or alien ones). In general, the body of *X. laevis* has a flattened shape. Adult males measure around 90 mm in males, and females 100 mm, although larger individuals are known (John Measey pers. comm. 2018). The skin is smooth and slippery, with peculiar lateral lines along its sides. The eyes are positioned at the top of a small head, which lacks a tongue and eardrums. The hind legs are very developed and webbed (with black claws on the first three toes), while the front limbs are rather small. Colour varies from yellowish to olive grey or dark brown with spots (but albino forms are also common in trade). Tadpoles are easily distinguished from other (native) anurans, particularly because of their distinctive barbells next to the mouth, mid-water suspension feeding and often near transparent, especially when small. Other *Xenopus* species may be found in the trade. Examples are *X. tropicalis* and *X. epitropicalis*, which Tinsley & McCoid (1996) considered regularly imported to Europe with tropical fish from West Africa (although the source seems a bit outdated in this context). However, there is no evidence about the occurrence of such species in the wild in the risk assessment area.
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	Some risk assessments exist for the species, i.e. for Great Britain (GB), USA, and Australia. In the GB the risk attributed to the species is low, with a medium level of uncertainty (NNSS 2011). According to the assessment "*X. laevis* was having very minimal impacts in the UK, given the very few populations occurring in the wild" (which in fact are considered currently extinct, see details below), and the reduced ability to reproduce and further spread in the country (even if climate change would facilitate this). The only concern would be the unknown impact related to the possible spread of diseases (including the chytrid fungus) to native amphibians, and of possible unforeseen scenarios due to future climate changes. However the conclusions of the GB risk assessment cannot be extended to other EU regions, given the different climate and habitat conditions, which are definitely more suitable for the species, e.g. in the Mediterranean area. In the USA an Ecological Risk Screening Summary was made by the U.S. Fish and Wildlife Service (Anonymous 2017). The overall risk assessment category attributed to the species is "high", as the climate matched well in many states, and the certainty of the assessment was deemed medium (given the taxonomic uncertainties noted for the target species). Similarly, in Australia *X. laevis* has been assigned an establishment risk rank of "extreme" (Page et al. 2008). Since these assessments focus on the occurrence of the species in the US and Australia, their validity is limited by the difference in ecological, geographical and climatic conditions compared to the EU situation. Moreover, in the case of the US, the assessment would probably should be revised in the light of the noted uncertainties (John Measey pers. comm. 2018).

	A number of studies aimed at ranking the impact of amphibians were also carried out at either the global level (e.g. Kumschick et al. 2017a, Kumschick et al. 2017b, Kraus 2015, Measey et al. 2016) or EU level (e.g. Kopecký et al. 2016). For example, Kumschick et al. (2017b) discussed the application of the Environmental Impact Classification for Alien Taxa (EICAT, see Hawkins et al. 2015 for details on the methodology) on amphibians following two independent assessments made by Kraus (2015) and Kumschick et al. (2017a). The results showed that the impact classification is relatively "high" for *X. laevis*, despite some minor difference between the two assessments: respectively Massive (MV, irreversible community-level changes) and Major (MR, impact on a native community that is reversible). This difference seems justified by the practical interpretation and assignment of disease impacts in the absence of direct evidence of transmission from alien to native species, especially in relation to chytridiomycosis. It is also worth mentioning that the SEICAT assessment found little documented evidence for socio-economic impacts, except in the species native range where it can be a predator in aquaculture (Bacher et al. 2018). Also Measey et al. (2016) used the generic impact scoring system (GISS) to carry out a global assessment of alien amphibian. In particular *X. laevis* was the second top scoring amphibian for impact on native ecosystems (considering the sum for environmental and economic scores together) only after the invasive Cane toad *Rhinella marina*. Finally, it is worth mentioning Kopecký et al. (2016) who applied a risk assessment model (RAM) to ornamental amphibians traded in the EU. *X. laevis* was used as a reference species (together with *L. catesbeianus*) and was considered to have a moderate risk (the RAM value is 0.365), with an AmphISK invasion score of 10 (on a scale -10 to 33). This system however does not provide overwhelming evidence of risk, because as pointed out by the authors the RAM establishment value cannot be viewed as a precise estimation of the probability of establishment, but rather provides a relative ranking of ornamental amphibians traded in the EU. As a general remark regarding the scoring of the impact discussed above, it is important to consider that the categories used here (see Annex II) do not fully match with those adopted, for instance, by EICAT (according to which a major impact is reversible, contrary to the definition in Annex II which consider a major impact as irreversible). This may explain some inconsistencies throughout the risk assessment. For example, as confirmed by John Measey (comm. per. 2018) on the data used for this assessment made by Kumschick et al. (2017b), *X. laevis* was scored Major (within the EICAT scheme) on the basis of two studies (i.e. Lillo et al 2011, Grosslet et al 2005) both regarding predation, which suggest ongoing decline

	in native species (both were given medium confidence). However, the same impact would be considered as Moderate according to guidance in Annex II.
A4. Where is the organism native?	The full range of *X. laevis* covers much of southern Africa: South Africa, Lesotho, Swaziland, Namibia, parts of Botswana, Zimbabwe, parts of Mozambique and extending north into Malawi (Measey 2016, Ihlow et al. 2016). It means that native populations are distributed from winter rainfall regions in the south-western Cape region to summer rainfall regions in the north; and from sea level to nearly 3,000 m in Lesotho (Measey 2004, De Busschere et al. 2016). As summarised by the Global Invasive Species Database (2015) *X. laevis* is a water-dependent species occurring in a very wide range of habitats, including heavily modified anthropogenic habitats. It lives in all sorts of water bodies, including streams, but tends to avoid large rivers, and water bodies with predatory fish. It reaches its highest densities in eutrophic water. It has very high reproductive potential. It is a highly opportunistic species, and colonizes newly created, apparently isolated, water bodies with apparent ease. *Xenopus laevis* exhibits high tolerance to salty water, pH variation (5-9, but there is evidence of the species breeding below pH 4, according to John Measey pers. comm. 2018), temperature variation (2-35+), and is capable of aestivation during dry periods. The species cannot spread naturally into the risk assessment area.
A5. What is the global non-native distribution of the organism outside the risk assessment area?	As reviewed by Measey et al. (2012) the global non-native distribution of the species is known to include four continents: North America, South America, Asia and Europe. In particular, established populations are present in different states of the USA, Chile, Japan. There are historic records also for Mexico, Java, Israel, and Ascension Island, but with the notable exception of Mexico (where the species occurrence was recently confirmed, see Peralta-García et al. 2014) the presence of the species in these countries is not confirmed. Regarding Europe, see details under points A7-A9 below.
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 species was recorded and is established in both the Mediterranean and Atlantic biogeographic regions.
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?	Current climate: Mediterranean, Atlantic, Continental and Alpine biogeographic regions Future climate: all biogeographic regions, with the exception of the Boreal and Arctic, may be suitable (at least in part) depending on the different models used (see ANNEX VII). However the confidence level is

	very low, as we were not able to retrieve precise information on future climate, given the methodological constraints and the lack of accurate information available on the species location and taxonomy. Recent studies have shown that native phylogeographic lineages have contributed differently to invasive *X. laevis* populations, but most of the introductions have probably been from the Mediterranean climate zone in the southwest of the Western Cape Province, South Africa, where *X. laevis* occurs naturally. For example, according to genetic and historical data the populations established in Europe, and in particular in Italy (Sicily), Portugal and France, seem to involve individuals from the south-western Cape region in South Africa (De Busschere et al. 2016, Lillo et al 2013). In France however another distinct native phylogeographic lineage is involved, i.e. from other regions of South Africa (De Busschere et al. 2016, Rödder et al. 2017). The identification of source populations is particularly relevant for the purpose of this document, because phenotypic as well as genotypic traits of colonizing individuals might influence the invasion process and success, particularly in such cases where there is extensive population differentiation within the native range (De Busschere et al. 2016). To assess the future distribution under current climate, Measey et al. (2012) used a single lineage of the species from the southwestern Cape of South Africa for their species distribution models (SDMs). As a result, the optimal uninvaded bioclimatic space was identified in isolated parts of France and Portugal only, while a large suitable climatic potential was identified for most of southern Portugal and adjoining Spain, as well as central and southern France, and mainland Italy. Such data are consistent with the finding of Ihlow et al. (2016), who used the entire range as well as invasive populations and who predicted particularly high probabilities in Europe, namely in Portugal, eastern Spain, southern France, and Italy. Furthermore, Ihlow et al. (2016) highlight areas in Spain (including the Balearic Islands), mainland Italy (including Sardinia), and southern France (including Corsica) to be highly vulnerable to potential invasions, as these regions exhibit suitable climatic conditions for *X. laevis* and are adjacent to established invasive populations. According to Measey et al. (2012) a few suitable areas were found also in the United Kingdom outside southern coastal areas, plus Greece, Ireland, Germany, Belgium, Denmark and the Netherlands (for details see maps developed by Measey at al. 2012). On the other hand, Ihlow et al. (2016) predict only moderate probability for Great Britain, where populations from Wales and Lincolnshire have recently been extirpated. Therefore, while the optimal area would fall within the Mediterranean and Atlantic biogeographic regions only, the maps annexed to the study seem to suggest the presence of suitable areas also within the Continental and Alpine regions. Under foreseeable climate change, using species distribution models (SDMs), Ihlow et al. (2016) assessed the global invasion potential of this species for 2070 following four IPCC scenarios (i.e. RCP2.6, RCP4.5,

	RCP6, RCP8.5). In particular, the potential range size was predicted to expand in north-western Europe, especially in France and Great Britain, where new regional conditions may promote new invasions or the spread of established invasive populations. The Mediterranean area was already considered suitable and still is under all climate change scenarios. The maps shown in the paper by Ihlow et al. (2016) do not allow for a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions in Europe will become suitable for the species. Rödder et al. (2017) demonstrated that invasive populations of *X. laevis* are established well beyond the species' multivariate realized niche in southern Africa. Hybridization of different lineages may have enabled a shift in the species' fundamental niche. Given the magnitude of the detected niche shifts, the usefulness of climate matching approaches to assess invasion risk for this species is challenged, as it might frequently underestimate the true potential distribution when a geographic subset of the species' realized distribution is used for model training. It can be expected that the true invasion potential for *X. laevis* is larger than its estimated potential distribution based on its currently realized niche (Rödder et al. 2017). For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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.	Recorded in the following Member States: France, Portugal, Italy, Spain, Sweden, Germany, Netherlands, UK. Established: France, Portugal, Italy. In general, the situation has been quite dynamic in the last years, mostly due to the fact that introductions are usually followed by a lag of around 15 years between the export of animals and the rise in invasive populations (van Sittert and Measey 2016). In general, it can take between 2 and 25 years or more for first reports of introductions to be released (Measey et al. 2012). As discussed in detail below, the species is currently considered established in France, Portugal and Italy (Sicily), but until recently the species was considered established also in the UK, where it is currently considered extinct. According to Frazer (1964) the first introduction in the UK occurred in Kent in 1955, but did not succeed. The UK was also home of the first invasive population established in Europe, namely on the Isle of Wight, due to an introduction around 1962 (Tinsley et al. 2015a, Tinsley & McCoid, 1996, van Sittert and Measey 2016). In the UK, in addition to the population on the Isle of Wight, now probably extinct, there have also been two established populations, namely in Glamorgan (Wales), and Lincolnshire (England). They were

	both the subject of an eradication programme and are considered recently extinct (Tinsley et al. 2015a) although follow-up monitoring is still required in South Wales (John Measey, pers. comm. 2018). The species was also reported in 1987 and 1990 in two ponds to the southeast of London, although these do not appear to have survived, and in the southwest of England, but no established populations have been found (see review made by NNSS 2011). In Portugal the species was first found in 2006 and first reported in 2007, but the first introduction may have occurred in Oeiras in 1979 (Sousa et al. 2018). In Italy, the only known population of *X. laevis* is on the island of Sicily where the date, site and cause of first release are all unknown (Measey et al. 2012). The first documented occurrence dates back to 1999, while the first report was in 2004. In peninsular Italy, the presence of two adults of *X. laevis* was reported in 2017 for the Lombardia region, in the Groane park (see http://www.parcogroane.it/wordpress/wp-content/uploads/2017/08/Report-censimento-anfibi-Progetto-LIFE-GESTIRE-2020-1.pdf). In France animals were officially first reported in 1998. However, residents of the area suggested that this frog had been present since the early 1980s (Fouquet 2001, Fouquet and Measey 2006, Louppe et al. 2017, Measey et al. 2012). In Spain, the presence of *X. laevis* was reported in Barcelona in 2007, but the species (apparently only larvae occurred there) was eradicated (Pascual et al. 2007). In Sweden, a single animal exists in the collection of the Gothenberg Natural History Museum collected in 2007 (Measey et al. 2012). However, this record is not reliable as the locality reported by Measey et al. (2012) is in fact a name of a person who received a dead frog from another person (Melanie Josefsson in litt. 2018). In Germany, *X. laevis* specimens have been collected in the Hamburg area, following a release in 1991 by animal rights activists (Tinsley and McCoid, 1996; Rabitsch et al., 2013). Their current status is unknown, but it is very likely that they have disappeared. In the Netherlands there are records of an adult individual caught near Gorichem in 1974 and of tadpoles collected near Utrecht in 1979 (Tinsley and McCoid, 1996).

	In Belgium, as reported in the Hyla database, 30 *X. laevis* larvae were found at Antwerp University pond in 2008 (observer Bart Vervust). Their current status is unknown. According to Measey (2017) wild caught *X. laevis* are also reported from in the Czech Republic and Switzerland, although such records are presumed anomalies and should be treated with suspicion, as no details or further data are known (it might be worth to remark that these are records from USFW for animals imported into the USA, hence they could be wild caught in South Africa and then move on, as pointed out by John Measey, pers. comm. 2018).
A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	Current climate: France, Portugal, Italy, Spain, Sweden, Germany, Netherlands, UK, Greece, Ireland, Germany, Belgium, Denmark, Netherlands Future climate: all EU countries may be suitable (at least in part) depending on the different model used (see ANNEX VII). However, the confidence level is very low, as we could not retrieve precise information on future climate, given the methodological constraints and the lack of accurate information available on the species location and taxonomy For details see comments on point A7.
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?	There is evidence of invasiveness in the USA, Chile and Japan (see section on impacts below for more details).
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	The species has shown signs of invasiveness in both the Mediterranean and Atlantic biogeographic regions, i.e. in all areas where populations are established. For details see section on “magnitude of impact”, points 2.13-2.30 below.
A12. In which EU member states has the species shown signs of invasiveness?	The species has shown signs of invasiveness in all EU Member States where populations are established, i.e. France, Portugal and Italy (Sicily).
A13. Describe any known socio-economic benefits of the organism.	The species is used as a pet and as a biological model for research (endocrinology, developmental biology, and reproduction, including anatomical studies and diagnosis of pregnancy). In relation to the pet sector, for some Member States and for the UK in particular, sales and associated ancillary product sales of *X. laevis* are significant. In the UK, such revenue is in the estimated range of between 168,500 euros to 3 million euros and this is likely to be a conservative estimate. This species is

	also likely to be economically important to other Member States where trade in this species (as a pet) is permitted, although EPO acknowledges that this may be to a lesser degree when compared to the UK. More in detail, according to EPO (2018) from the data from those Member States where *X. laevis* is traded by the pet sector, individual animals are sold for values between 1 euro to 11,30 euros depending on the MS. Therefore, in terms of the total trade based on the information collated by EPO, there is a very broad range in the trade values of this species (based on the number of individuals sold), with numbers ranging from a lowest value of 275 euros per annum to as high as 1 million euros per annum. If ancillary products e.g. aquariums, dry goods etc. are then factored in, this figure is significantly higher with a conservative estimate of 3 million euros. It should be noted however, that the proportion of sales of animals (and therefore ancillary products) varies significantly between Member States, with the UK representing the highest values whilst those reported for the Netherlands and France being significantly lower. As per previous comment above, due to the skewed nature of the raw data (which EPO was unable to provide given that it is highly commercially sensitive), EPO unable to provide median or average estimated trade revenues. However, EPO noted that the figures provided are likely to be a conservative estimate. In the UK, anywhere between 168,500 euros to 3 million euros. This represents a very broad range, which means that banning this species could have an impact for pet stores related activities in some EU countries. In relation to the species being used as a biological model for research, as pointed out by van Sittert and Measey (2016) this frog had a significant role in the history of 20th century science as it became one of four vertebrate species universally recognised as representing a standard biological model of all vertebrates (van Sittert and Measey 2016, Gurdon and Hopwood, 2000). Data from Measey (2017) show that there is some trade to the US from other countries with known invasive populations (UK and France), but in small quantities (< 100 animals), and of captive bred animals usually for medical or scientific purposes and are thus presumably not from invasive populations. Indeed, *Xenopus* suppliers exist in the risk assessment area, e.g. in France and UK (see http://www.xenbase.org/other/obtain.do). For example, animals in France were being harvested by *Xenopus* Express in France (John Measey, pers. comm. 2018). However, according to data presented in Measey (2017), live individuals of *X. laevis* were imported to the USA also from other EU countries, such as Czech Republic, Germany, and Italy. No detailed information was available on the actual socio-economic benefit of this species in relation to research activities, hence the potential impact of any relevant suspension of its trade and use is to be considered although is not quantifiable. On the other hand, it is possible that any discontinuation of the use of this species, could lead to the risk of the animals (either adults or tadpoles) being released/dumped in the environment.

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."
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	**few**	**high**	In Europe, the occurrence of *X. laevis* in the wild is thought to be a consequence of its use as a research model in laboratories and as a pet (Measey et al. 2012, Tinsley et al. 2015a). The main active pathways are therefore the following:

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

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

			1) Pet/aquarium/terrarium species (escape from confinement); 2) Research and ex-situ breeding (in facilities). As pointed out by Tinsley & McCoid (1996), it may be due to many factors, such as loss of interest, end of an experiment, misguided ethics or curiosity, which occasionally results in the release of captives. High rates of deliberate release are reported, along with aquaculture escapes (Measey et al. 2012). However, in most cases the exact cause is only inferred retrospectively, as the species is often detected only many years after its deliberate or accidental introduction. For example, in Portugal the species lived undetected for more than 20 years (Sousa et al. 2018). In such cases, it is clear that it is not possible to establish the intentionality of the introduction without the relevant events being appropriately documented).
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	1) Pet/aquarium/terrarium species (escape from confinement) 2) Research and ex-situ breeding (in facilities)		In addition to the use of the species as a research model in laboratories and as a pet (which leads to the main two active pathways already identified), other uses are known. For example, the species has been used in schools for training in labs (e.g. dissections etc.), which can be a source of animals released in the wild. While there is no documented evidence of such releases in Europe, in the US, schools are known to ditch their stock when legislation changed making keeping invasive species illegal. The law changed without making any provision for people already keeping them (John Measey, pers. comm. 2018). In any case, the use of animals in schools

			is treated here under the pathway "Research and ex-situ breeding (in facilities)", see point 1.4b below. As reported by Weldon et al. (2007) in South Africa *X. laevis* is appreciated as live bait for freshwater angling (despite this practice being illegal). As a consequence, fishermen are known to seed dams with *X. laevis* in order to produce a local supply of live bait (Measey et al. 2017). However, this is not considered an active pathway in Europe. Zoo exhibit trade of this species is also mentioned as a former pathway in the late 1900s (Vredenburg et al. 2013), and although it is still present in public zoos/aquaria, is not considered as an active pathway in Europe. Whilst there is no data available on the total population within all zoological collections in 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 exceptions of Cyprus and Malta). The information provided by EAZA (EAZA, 2018 pers. comm. through a document circulated by email by the EC on 25/07/2018) indicates that 84 specimens in total are kept by 15 zoo/aquarium EAZA Members in 11 Member States (BE, UK, HU, DK, NL, IE, DE, PT, PL, EE, FR). On top of this, in total 5 specimens of the subspecies *Xenopus laevis laevis* are kept by 1 zoos/aquariums EAZA Members in 1 Member States (CZ). 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 the actual situation

			might slightly differ if the species has been recorded under a different/older taxonomic name (as in the case of *X. l. laevis*).
Pathway name:	**1) Pet/aquarium/terrarium species (escape from confinement)**		
1.3a. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	**intentional**	**high**	The species is traded as pet, and as such the introduction in the risk assessment area through this pathway is intentional. However, the entry into the environment is either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes. Despite the general lack of documented evidences regarding the exact pathway of introduction for this species, there are clues of this pathway being active in Europe, as well as in other parts of the world. For example, according to Measey et al. (2012) the source of a population once occurring near Scunthorpe, Humberside, in the north-east of England, is thought to be due to the closure of a pet shop and the deliberate release of adults in the mid-1990s. *X. laevis* had been and was still sold at local pet shops in Portugal at the time of the publication of the study by Rebelo et al. (2010). According to the European Pet Organization (EPO 2018) *X. laevis* is traded by the pet sector in several Member States of the EU although this species appears to be subject to diverse national measures in different Member States. It is forbidden to sell it freely in France, but in the UK and Belgium pet stores can still sell this species. In France, in order to be allowed to keep *X. laevis*, professionals need a competency certificate and an opening authorization issued by local authorities. This means that only a handful of professionals are authorized

			to keep *X. laevis*. In terms of import into the EU, some smaller animals are imported to the UK from the Czech Republic whilst it is also imported to and traded from the Netherlands. According to EPO (2018) larger specimens of *X. laevis* are available from UK breeders also in relation to the pet sector (according to EPO, the albino morph of *X. laevis* is predominantly traded within this sector). As a side note, also in Chile there is also evidence of continuous releases which helped the species spread, and which are presumed to be connected with the use of animals in the pet trade, as private owners were suspected of dumping them (Lobos & Jaksic 2005, Measey et al. 2012). Also in the USA, there is evidence of animal importer dumping unwanted stock, e.g. in Florida (King and Krakauer 1966), or intentionally released by a single person, in Arizona (Somma 2018, Tinsley and McCoid, 1996).
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	**very likely**	**high**	In general, information on the origin of animals, exact number of individuals in trade and those in captivity, is not available. Also, no information could be retrieved on number of introduction events and number of individuals (either adults or larvae) escaped and/or released in the environment, hence it is not possible to assess the propagule pressure. However, according to the European Pet Organization (EPO 2018) in relation to animal traded in the UK, *X. laevis* is captive bred and none are wild caught. The extent of breeding in other EU Member States is unknown. In terms of numbers traded, EPO has limited quantitative data based on several Member States with a wide range of volumes. The data that EPO is able to provide (EPO is unable to provide the raw data given that

			this information is commercially sensitive) suggest a widespread range with the lowest value being 100 animals sold per annum to the highest value of tens of thousands of animals being sold per annum. Therefore, due to this wides range of values, EPO is unable to provide median or average numbers/volumes due to the skewed nature of the raw data. Other considerations may help to figure out the dimension of the problem. For example, as documented in the USA, it is worth mentioning that across the last decades *X. laevis* trade has changed dramatically in terms of primary purpose, frogs' origin and numbers of animals traded. The species was originally distributed for pregnancy testing and laboratory use, but in the last 15 years, the size of the trade for medicine and science dropped to only 0.1% of imports, with the pet trade commanding 99.6% (Measey 2017). Trade figures reported by Measey (2017) for the USA are impressive (see also Herrel and van der Meijden, 2014). Whilst trade for medical and scientific purposes is now minimal (a few hundred animals per year), the pet trade imported 1.83 million live animals over the last 15 years (a total of 1,856 shipments which ranged from single animals to 11.5 thousand individuals). Just to give an idea of the global trade network supporting such trade, 75% of these animals are imported from Hong Kong (although it is possible that many animals originate from mainland China or elsewhere). It's also worth noting that all of these Chinese animals appear to be albinos, and there are currently no published reports of invasive albino populations, despite a single exception recently found in China (John Measey, pers. comm. 2018, Wang et al. sub). However, further research is required to confirm this (John Measey, pers. comm. 2018). Only 5,600

			animals were imported from the native area in South Africa, and this trade ceased in 2003. Nearly 200,000 individuals were imported from Chile and the majority of these were reported as being wild caught, suggesting that the invasive population there is being exported for the US pet trade (Measey 2017). Given the supposed wide distribution of this species in the pet trade in several countries, the risk of reinvasion after eradication is to be considered as likely as a first introduction. There are no specific studies providing an indication of the propagule pressure, but single gravid females can contain from 1,000 to 27,000 eggs per clutch, and they will produce multiple clutches in a season under favourable conditions (Global Invasive Species Database 2015), therefore even a handful of individuals may be sufficient to start a new population. In fact, as shown by Lobos et al. (2014), the invasion of *X. laevis* in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population. Also according to Measey et al. (2012) propagule pressure plays a pivotal role in the establishment of *X. laevis*, as some populations became established after the release of large numbers of animals from breeding facilities (laboratory and pet supplies). Other evidence of populations that have established from very few individuals is not available (John Measey, pers. comm. 2018).
1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)?	**very likely**	**medium**	The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been frequently traded and that the origin of some animals

Subnote: In your comment consider whether the organism could multiply along the pathway.			successfully released in the wild is attributed to this pathway.
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	**very likely**	**low**	No information has been found. Trade is intentional, and as such there is no management practice in place to prevent the species entering the risk assessment area. Also, there are no known specific practices for preventing this species from escaping or being released in the wild.
1.7a. How likely is the organism to enter the risk assessment area undetected?	**very likely**	**high**	While animals intentionally introduced in the risk assessment area for the pet trade are clearly not at all undetected, those being introduced in the wild as a consequence of accidental escapes or intentional releases can be undetected for many years (see point 2.7a below)
1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**moderately likely**	**medium**	We are not certain whether any particular time of the year is more appropriate for establishment. It is likely that *X. laevis* could establish during any month of the year. In any case, traded animals may arrive and be released or escape at any time during the year in Europe, but data about frequency and months of the year are unknown
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**moderately likely**	**medium**	It is likely that people who deliberately release *X. laevis* into the wild will do it in what they consider the most suitable habitat. As a remark, *X. laevis* is a vigorous adaptable species which may virtually inhabit any type of water bodies, including lakes and rivers, as well as permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides *X. laevis* thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005). This clearly increases the likelihood of the species being introduced, either intentionally or accidentally, into suitable habitats. There

			is also evidence that additional translocations by humans within the risk assessment area may occur, hence increasing the opportunities for the species introduction within the risk assessment area. In addition, as pointed out by Measey et al. (2012) biosecurity at breeding facilities is clearly of paramount importance, but the maintenance staff in the pet trade may not have appropriate information or relevant training.
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**very likely**	**high**	In current conditions, the overall likelihood of entry into the EU based on this pathway is very high. The species is known to be present in the pet trade in Europe, and has already been recorded in the wild in the region, possibly also as a consequence of this pathway.

Pathway name:	**2) Research and ex-situ breeding (in facilities)**		
1.3b. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	**intentional**	**high**	The species is traded as model amphibian in scientific research, and as such the introduction in the risk assessment area through this pathway is intentional. However, entry into the environment is either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes. Despite the general lack of documented evidences regarding the exact pathway of introduction for this species, there are clues of this pathway being active in Europe, as well as in other parts of the world. For example, in France the suspected origin of the species was a breeding facility of the CNRS in Fronteau, Bouillé St Paul (Fouquet 2001), a laboratory supplier for French research institutions (Measey et al. 2012). In Portugal, the species was likely introduced following a flood of the 1979/1980 winter in

			the laboratories, where the species was used, although this is unconfirmed (Sousa et al. 2018).
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	**very likely**	**high**	There is evidence linking the occurrence of invasive alien populations with the trade and use of this species for biomedical research, although other secondary pathways seem to be involved as well (van Sittert & Measey 2016). The history of the use and trade of the species started in the 1930s with the use in pregnancy testing until the 1960s, and later for laboratory use as model organism (Gurdon and Hopwood 2000, Measey et al. 2012, Tinsley et al. 2015a, van Sittert & Measey 2016). This led to exports of thousands of live animals from its native South African Cape region to laboratories, first to the United Kingdom and eventually all over the world. By 1970, as demonstrated by van Sittert & Measey (2016), *X. laevis* was the world's most widely distributed amphibian: institutions in 48 countries were supplied with live animals on all continents except Antarctica. In fact, as summarized by Weldon et al. (2007), the use of this species as a model amphibian in scientific research (i.e. genetics, molecular biology, embryology, biochemistry and ecotoxicology) was increasingly popular in the 1970s, and *X. laevis* became the most widely used amphibian in research in the 1990s. In terms of numbers, over 10,000 animals were exported annually from South Africa between 1998 and 2004 to 132 facilities situated in 30 countries (Weldon et al. 2007). However, there is still no known information on how many animals were shipped privately and where they were shipped to, during this period. Additionally, the secondary movement between places that were supplied also appears to be important (John Measey, pers. comm. 2018).

			X. laevis was also used for educational and training purposes in schools and universities (e.g. dissection classes). However, this use seems declining markedly due to ethical concerns and financial constraints (Reed 2005). Of note here is the link between tadpoles and home teaching: in many cases, the tadpoles are reared in large numbers and then many are euthanized. Some individuals will give tadpoles over to parents to raise them at home, or liberate tadpoles. This is less likely in the pet trade, but may happen in tertiary education institutes where *X. laevis* is a teaching model (John Measey, pers. comm. 2018). Given the supposed wide distribution of this species in research facilities in several countries, the risk of reinvasion after eradication is to be considered as likely as a first introduction. See also comments on point 1.4a.
1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**very likely**	**medium**	The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been frequently used as a research model and that the origin of some animals successfully released in the wild is attributed to this pathway.
1.6b. How likely is the organism to survive existing management practices during passage along the pathway?	**very likely**	**low**	No information has been found. Movements of animals for their use in research activities is intentional, and as such there is no management practice in place to prevent the species entering the risk assessment area. There are no known specific practices for preventing this species from escaping or being released in the wild.
1.7b. How likely is the organism to enter the risk assessment area undetected?	**very likely**	**high**	While animals intentionally introduced in the risk assessment area for use in research activities are clearly

			not at all undetected, those being introduced in the wild as a consequence of accidental escapes or intentional releases can be undetected for many years (see point 2.7a below).
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**moderately likely**	**medium**	We are not certain whether any particular time of the year is more appropriate for establishment. It is likely that *X. laevis* could establish during any month of the year. In any case, traded animals may arrive and be released or escape at any time during the year in Europe, but data about frequency and months of the year are unknown.
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**moderately likely**	**medium**	As pointed out in the GB risk assessment for the species (NNSS 2011), most of the African clawed frogs that are present in captivity in the UK are owned by commercial laboratories, which will be careful to prevent escapes. These laboratories are generally run by competent people who have an interest in amphibians and who realise the negative consequences of releasing these animals into the wild. Measey et al. (2012) seem less optimistic, as they recognise that biosecurity at breeding facilities is clearly of paramount importance, but the maintenance staff in laboratories may not have appropriate information or relevant training. In fact according to Measey et al. (2012) in at least one case tadpoles of *X. laevis* were released routinely for many years into a pond of university property, despite the fact that the person releasing these tadpoles was instructed to euthanise them. In another case, tadpoles had been given to local schools and friends for learning purposes (Measey et al. 2012). Laboratory security has been increasingly improved over the years so escapes are now very unlikely (NNSS 2011). However, people working with alien species (even when in special facilities) should be made aware of the issues concerning release (John Measey, pers. comm. 2018).

			In conclusion the likelihood of the species being intentionally released in the wild, in a suitable habitat, should be low. However, there is always the risk of unexpected events which may cause the escape of the animals, as was the case in Portugal where a laboratory experienced flooding, although this pathway was not confirmed (Measey et al. 2012). There is also evidence that additional translocations by humans within the risk assessment area may occur, hence increasing the opportunities for the species introduction within the risk assessment area. As a remark, Measey et al. (2012) expressed concern about the future risk of laboratory populations of *X. laevis* which - due to the possible replacement of this species with *X. tropicalis* as a research model organism - may be dismissed and dumped in the environment (which is not explicitly stated, although it seems what the authors imply). In fact, although there are no documented instances with respect to academic replacement of model organisms, there are examples of this in the US where school pets became illegal to keep and animals were dumped (John Measey, pers. comm. 2018). Given the instruction to euthanize a large number of animals, many people will still choose to dump living animals into the natural environment, when they don't have specific knowledge of what might happen (John Measey, pers. comm. 2018).
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**very likely**	**high**	In current conditions, the overall likelihood of entry into the EU based on this pathway is very high. The species is already present in research facilities in Europe, and has already been recorded in the wild in the risk assessment region, possibly as a consequence of this pathway.

End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	**very likely**	**high**	The overall likelihood of entry into the risk assessment area based on all pathways is very high in current conditions, particularly given the fact that the species is present in trade and in breeding facilities in many countries, possibly in all biogeographical regions.
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	**very likely**	**high**	As reported by Tinsley et al. (2015a) the species originates from Western Cape, South Africa, and has been introduced on four continents, mostly in areas with a similar Mediterranean climate, but also in cooler environments (where persistence for many decades suggests a capacity for long-term adaptation). This suggests that recent climate warming might enhance invasion ability, favouring range expansion, population growth and negative effects on native faunas (Tinsley et al. 2015a). The introductions occurring well out of the Mediterranean climate zone, show the risk that an increasing number of invasions may occur, and that these aren't reported in the literature very quickly (John Measey, pers. comm. 2018). In fact, under foreseeable climate change, the global invasion potential of this species for 2070 assessed by Ihlow et al. (2016) following four IPCC scenarios (i.e. RCP2.6, RCP4.5, RCP6, RCP8.5) may expand in north-western Europe and the Mediterranean area. In fact recent studies show that invasive populations of *X. laevis* are established well beyond the species' multivariate realized niche in southern Africa (Rödder et al. 2017). The maps shown in the paper by Ihlow et al. (2016) and Rödder et al. (2017) do not allow a precise identification of the biogeographic regions where the species could

			establish in the future under foreseeable climate change. However, it seems that most biogeographic regions may become suitable for the species. An improved mechanistic model to describe the likely extension of the range of this species (looking also at the physiology of tadpoles and the consequences of breeding between lineages from different regions) is being built (John Measey, pers. comm. 2018). In conclusion, the likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change condition is likely to be the same as in current conditions (see above). However, no documented evidence exists to support this statement.

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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	**very likely**	**high**	According to Measey et al. (2012) a large suitable climatic potential was identified for most of southern Portugal and adjoining Spain, as well as central and southern France, and mainland Italy. Such data are consistent with the findings of Ihlow et al. (2016), who highlighted areas in the main Mediterranean islands (namely the Balearic Islands, Sardinia, and Corsica) to be highly vulnerable to potential invasions. According to Measey et al. (2012), a few suitable areas were found in the United Kingdom outside southern coastal areas, plus Greece, Ireland, Germany, Belgium, Denmark and the Netherlands (for details see maps developed by Measey at al. 2012). Ihlow et al. (2016) predict only moderate probability for Great Britain. Despite this, the species has had persistent populations in the UK that are now exinct.
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	**very likely**	**high**	*X. laevis* is a vigorous adaptable species which may virtually inhabit any type of water bodies, including lakes and rivers, as well as permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides that, *X. laevis* thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005). These

			habitats are widespread all over the EU, including countries where the species is not yet established. Additionally, Moreira et al (2017) have recently documented that this species can breed in both lotic and lentic environments. The use of lotic habitats may open up even more habitats for breeding, and may contribute to the maintenance of the invasive population even in the absence of lentic sites.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	**widespread**	**high**	*Xenopus* species in sub-Saharan Africa inhabit virtually all water bodies, including large rivers and lakes, as well as permanent and temporary ponds over a wide range of altitudes and temperatures (Measey 1998). According to John Measey (pers. comm. 2018), animals are often found in very low abundance (and occasionally very high) in natural systems, but numbers can become overwhelming in in modified habitats. The latter are normally enriched and eutrophic, which probably helps build up their numbers. Measey et al. (2012) pointed out that comparatively few reports exist of *X. laevis* in its natural habitat, hence the lack of knowledge about the native ecology and natural dispersal of this globally invasive species. However, there are a few studies on the invasive range which provide useful information on this regard. For example, according to a study on habitat suitability carried out in Chile, Lobos et al. (2013) confirm that lentic aquatic environments, with slow drainage and murky waters, highly connected, human-disturbed, and part of an irrigation system of small streams and canals, account for the highest probabilities of successful establishment of *X. laevis* within the area of invasion. As reported by Lobos & Jaksic (2005) *X. laevis* in Chile lives from almost sea level up to 620m, and inhabit quite a diverse array of habitats

			with regard to water temperature, dissolved oxygen, pH, and electric conductivity, indicating a high degree of adaptability and colonization potential. It is also worth remarking that animals are also found in ponds that are unconnected like in France and Sicily, which could mean that *X. laevis* is able to move overland (John Measey pers. comm. 2018), although the possible contribution of further man-mediated releases should be taken into account in these cases. A study by Moreira et al (2017) in Portugal documented that *X. laevis* breeds in small streams and ponds, suggesting that while lentic sites are most likely responsible for population booms, the potential reproduction in lotic sites may contribute to the maintenance of the invasive population even in the absence of lentic sites. There are numerous ponds, lakes and other water bodies that are potentially suitable for the survival, development and multiplication of this species in the EU.
1.16. 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 ?	**NA**		Not relevant to this species (NNSS 2011).
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	**very likely**	**high**	*X. laevis* has only a few competitors that may prevent its establishment in the EU, the most effective being fish (e.g. eels), but the species may find suitable habitats where such competitors are absent (see Tinsley et al. 2015a). According to John Measey (pers. comm. 2018) fish appear to influence where animals will colonise, and it is possible that

			this could be used to prevent further invasions (e.g. a ring of ponds with introduced fish). Other non-native competitors are mentioned by Prinsloo et al. (1981), i.e. the Chinese silver carp, *Hypophthalmichthys molitrix*, as both the tadpoles and the silver carp compete for phytoplankton as food, and the Chinese bighead carp *Aristichthys nobilis* (zooplankton feeder). *Xenopus laevis* may live in the same ponds as crabs and terrapins, and undergo significant predation and mutilation from these groups, but without moving (John Measey, pers. comm. 2018).
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	**very likely**	**high**	In Africa, *X. laevis* have evolved morphological, behavioural, and biochemical predator avoidance strategies, and in extralimital situations, for example in California, it is likely that predatory pressure is considerably reduced (for example by occupying sites lacking predatory fish), thus contributing to the success and spread of *X. laevis* (McCoid and Fritts, 1980). Despite the lack of dedicated studies on the issue, the situation in the EU may be similar (as demonstrated also by the successful spread of the species in some countries). In principle, *X. laevis* can be a prey for several species, including fish, snakes, birds and mammals. For example, in the UK, *X. laevis* may be eaten by typical fish and amphibian predators including herons, American mink (*Neovison vison*) and, possibly, grass snakes (*Natrix helvetica*). Eels are potential predators too (Tinsley et al. 2015a). During a study in western France, Eggert and Fouquet (2006) showed that predation by the

			polecat (*Mustela putorius*) was deemed the major adult mortality factor, together with (assumed) freezing. In its native range in South Africa, *X. laevis* is eaten by large fish, turtles, frogs, snakes, aquatic insects, and birds (Lafferty & Page, 1997). This list is actually far longer. Almost every predator eats the adults, crabs eat the eggs and larvae, and odonates and fish eat the tadpoles (John Measey, pers. comm. 2018). Similarly, in its introduced range outside the EU, i.e. in Chile, three bird species were observed to prey on *X. laevis*: Night heron (*Nycticorax nycticorax*), Kelp gull (*Larus dominicanus*) and Burrowing owl (*Speotyto cunicularia*) (Lobos & Jaksic (2005). In the USA *X. laevis* is preyed upon by Two-striped Garter Snakes (*Thamnophis hammondii*). Large fish, and the American Bullfrog, (*Lithobates catesbeianus*) are considered to be potential predators as well (Lafferty & Page, 1997). Additionally, according to Prinsloo et al. (1981), the largemouth bass (*Micropterus salmoides*) is a known “biological control” against *X. laevis*. Regarding parasites and pathogens, *X. laevis* carries a rather diverse parasite load, and does not seem to be particularly affected by any of them. In Portugal for example, this species was found to be infected by autochthonous parasites, probably proceeding from *Pelophylax perezi* (Rodrigues 2014). However the parasite burden was not as high as in the species they co-exist with, or as high as in the habitats where it is native, which in fact could enable this

			species to dominate the streams where it was recently introduced.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	**very likely**	**low**	There are no specific management practices in place in the EU which may prevent the organism from establishing wild populations (as demonstrated by the successful establishment of the species in some countries). The release of fish (e.g. for sport and angling) which may predate on *X. laevis*, like the non-native largemouth bass (*Micropterus salmoides*) is a noteworthy exception. However, the species may still occupy sites lacking predatory fish.
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	**moderately likely**	**low**	Overall, the species is known to thrive in highly disturbed habitats. For example, in southern California this is considered a common factor in all established populations (McCoid and Fritts, 1980). Therefore it is likely that management of water bodies facilitates the establishment of the species by contributing to the creation of suitable habitats. However there are opposite views. For example, management of UK water bodies and connecting habitats tends to be more intense than in many parts of the natural range of *X. laevis* which would in fact be more likely to hinder, rather than aid, establishment (NNSS 2011).
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	**very unlikely**	**high**	The species likely went likely extinct by natural means in the UK. However, eradication at a few sites (Measey et al. 2012) using a deliberate, targeted eradication campaign to eliminate the species fairly rapidly was considered a possibility, although follow-up surveys and control measures would be necessary (NNSS 2011).

			Small eradication campaigns were carried out successfully in the UK, Spain, and the USA (Measey et al. 2012) but in general this was only possible in small areas and at an early stage of invasion. The chances of success seem related more to the specificities of the water bodies affected (e.g. type, size, and overall network) rather than to the biological properties of the species. For example, care must be taken about when this is done as individuals are capable of surviving in the ground for many months (John Measey, pers. comm. 2018). Otherwise it is clear that the appropriate methodologies need to be identified carefully in relation to the species biological properties. For example, high concentrations of Rotenone failed to eradicate clawed frogs in Los Angeles County (St. Amant, 1975), because clawed frogs are air breathers (McCoid and Fritts, 1980).
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	**very likely**	**high**	There are no specific studies providing an indication of the propagule pressure, but a single gravid female can contain from 1,000 to 27,000 eggs per clutch, and will produce multiple clutches in a season under favourable conditions (Global Invasive Species Database 2015), therefore in principle only a handful of individuals may be sufficient to start a new population. As shown by Lobos et al. (2014), the invasion of *X. laevis* in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population (although the number of released frogs is unknown, see Lobos and Jaksic 2005). According to Measey et al. (2012) propagule pressure plays a pivotal role in the

			establishment of *X. laevis*, as some populations became established after the release of large numbers of animals from breeding facilities (laboratory and pet supplies). Also De Villiers et al (2016) found that small numbers of *X. laevis* can produce hundreds of adults within relatively short periods (e.g. 18 months). *X. laevis* is principally aquatic throughout its life. In general, tadpoles take 3 months to metamorphosis, and sexual maturity is achieved within one year (Global Invasive Species Database 2015) although this may happen only in certain circumstances (i.e. this was in California and may even be greater than in its native range in South Africa according to John Measey, pers. comm. 2018). Field studies by Tinsley et al. (2015a) showed that in favourable conditions there may be good recruitment, fast individual growth rates and large body size; maximum longevity exceeds 23 years. After all, the reproductive biology of the species seems very flexible. For example in its alien range in the US *X. laevis* reproduction reportedly occurred in most months of the year, in contrast to the shorter breeding season in South Africa (McCoid and Fritts, 1980). Also in its native range in South Africa, where the breeding season is poorly reported, seems to cover two distinct areas where the breeding times are opposite (John Measey, pers. comm. 2018). In countries like the UK, *X. laevis* appeared not to breed prolifically under current climatic conditions, but a large recruitment event was considered possible should suitable weather conditions occur

			for even one season within the period covered by the occurrence of this species in the wild (NNSS 2011).
1.23. How likely is the adaptability of the organism to facilitate its establishment?	**very likely**	**high**	As summarized by Measey et al. (2012) *X. laevis* is characterized by a suite of physiological and behavioural traits which makes this anuran very robust and versatile, enabling *it* to cope with dehydration, high levels of salinity, starvation and anoxic conditions. Both adults and larvae perform well over a wide range of temperatures, and larvae can metamorphose in a wide range of temperatures. Behavioural traits include their capability to migrate overland, to survive drought by burrowing into drying mud and to starve for up to 12 months (see Measey et al. 2012, Tinsley et al. 2015a). For example, during drought in the UK, *X. laevis* could survive in isolated pools in the river course, in subterranean water bodies and by burying themselves in mud (Tinsley et al. 2015a). Additionally, *X. laevis* shows specific adaptations to aquatic life, including retention of the lateral line system in adults, aquatic chemoreceptors and a body structure particularly adapted for swimming (Elepfandt 1996). *X. laevis* is a very adaptable species, which may virtually inhabit any type of waterbody, including lakes and rivers, as well as permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides *X. laevis* thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005).

			After all, as pointed out by Tinsley & McCoid (1996), the hardiness which has made *X. laevis* ideal for laboratory maintenance, has proved to be a considerable advantage for adaptation to new environments. Recent studies show that invasive populations of *X. laevis* are established well beyond the species' multivariate realized niche in southern Africa (Rödder et al. 2017). As pointed out by John Measey (pers. comm. 2018) it is worth considering that the native range of *X. laevis* is tropical to Mediterranean, hence from arid desert areas to high rainfall zones, and from sea level to 3000 m asl: this encompasses a massive climatic envelope but does not include their fundamental niche which is likely to have been much larger at the LGM. In addition, the broad global trophic niche of *X. laevis* and its ability to adapt its diet according to local conditions further contribute to the strong invasive potential of this species (Courant et al. 2017a). The results of the study by Measey et al. (2016) indicate that no prey categories are strongly selected for, suggesting that *X. laevis* does not usually specializes its diet and hence does not develop a population specific dietary niche. This characteristic may enhance its capacity to establish and spread in novel environments. Furthermore, field data confirm that adults may rely on their own offspring as a food source, enabling older individuals to survive periods of food shortage by exploiting the algal populations eaten by their tadpoles (Tinsley and McCoid 1996), although tadpoles are limited to a certain niche by being obligate suspension feeders (John Measey, pers.

			comm. 2018).
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	**likely**	**high**	As shown by a study by Lobos et al. (2014), the invasion of *X. laevis* in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population (although such low diversity may be not as meaningful as claimed as the study focused on mtDNA, according to John Measey, pers. comm. 2018). Therefore, low genetic diversity is not expected to be a problem for the species invasion process. It is also worth mentioning that these are tetraploid animals, and that this may mitigate against potential bottlenecks (John Measey, pers. comm. 2018).
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	**very likely**	**high**	The species has already shown to be able to successfully establish viable populations in the risk assessment area, e.g. in Portugal, France and Italy.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	**likely**	**medium**	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 (like happened in the past and led to the occurrence of the populations recorded in the risk assessment area and beyond).
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	**very likely**	**high**	According to the studies carried out by Measey et al. (2012) and Ihlow et al. (2016) suitable areas (plus some limited optimal areas) fall within the Mediterranean and Atlantic biogeographic regions, as well as the Continental and Alpine regions. Established populations are already present in the former, but not in the latter.

1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	**very likely**	**high**	Under foreseeable climate change, the global invasion potential of this species in 2070 assessed by Ihlow et al. (2016) following four IPCC scenarios (i.e. RCP2.6, RCP4.5, RCP6, RCP8.5) may expand into northwestern Europe and the Mediterranean area. The maps shown in the paper by Ihlow et al. (2016) do not allow a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions may become suitable for the species.

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	**moderate**	**high**	As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and rivers as migration corridors, but also performs terrestrial migrations (even without rainfall). It should be noted, however, that movement through streams and irrigation channels appears to be much faster than overland movement (Fouquet and Measey 2006). In a study that compared *X. laevis* invading an urban area with 2 other species, Vimercati et al (2017) suggested that they may be slower, but build up densities much higher and are arduous to detect. Indicative figures of estimated rate of dispersal are available from a few studies in both the species native and alien range. For example, estimated annual spread of feral populations varied between 1 km in France (Fouquet and Measey 2006) and 5.4 km in Chile (Lobos & Jaksic 2005). In particular, a study by Fouquet and Measey (2006) in France showed that, while lotic corridors are used by this principally aquatic species, most ponds are colonised through overland migration. According to Fouquet and Measey (2006) *X. laevis* is able to detect

			the presence of non-colonised ponds at a distance, and orient towards them. According to Fouquet and Measey (2006) the terrestrial spread can be estimated at approximately 1 km per year. In Italy, although the rate of spread is not sufficiently assessed, observations were made of newly colonized ponds at a distance of between 400 and 700 m from the nearest pond occupied by *X. laevis*, where it is likely that most individuals disperse overland (irrigation ditches are not present in the area and the ponds are not connected with each other) or are facilitated by a few temporary streams (Measey et al. 2012). Natural spread in the UK appears to have been very slow or non-existent (NNSS 2011) but animals occurred in a very particular system, and spread out of this area would have been very difficult (John Measey, pers. comm. 2018). Additionally, Measey and Tinsley (1998) reported a female travelling 0.2 km in 48 hours. Overland dispersal rates appear to be slower, compared to situations with ponds close to downstream dispersal corridors (Measey et al. 2012), but as reported by both Faraone et al. (2008) and Fouquet and Measey (2006), population dispersal by terrestrial movement seems prevalent in Italy and France. In particular, in France overland movements of 0.5 km per year are reported (Grosselet et al. 2006), and an adult female followed by radio telemetry moved overland 80 m from a pond through a pasture, crossing a wooded hedge and reaching a puddle 20 centimetres deep (Eggert and Fouquet 2006). According to Measey (2016) distances moved overland were from 40 m to 2 km (although the 2 km distance could have included use of a river), which is comparable to distances travelled by other terrestrial amphibians. There is no apparent difference

			between native and invasive ranges, besides, walls and thick vegetation are regularly traversed). In fact, in native populations in South Africa a female moved over 2.3 km in less than 6 weeks (De Villiers and Measey 2017). Louppe et al. (2017) found differences in mobility at the range edge of an expanding invasive population of *X. laevis* in the west of France; in particular individuals from the range edge had a greater stamina and had longer legs compared to individuals at the centre of the range, suggesting fast evolutionary optimization of dispersal abilities. This of course may have implications for conservation because spatial sorting on the range edge resulting in the evolution of locomotory capacity may lead to an accelerated increase in the spread of this invasive species in France. Also, Courant et al. (2017b) found that the level of resources allocated to reproduction was lower at the periphery of the colonized range compared to the centre and may be the result of changes in trade-offs between life-history traits. Such a pattern could be explained by interspecific competition or enhanced investment in dispersal capacity. There is evidence that additional translocations by humans within the risk assessment area may occur, however intentional anthropogenic "spread" via release or escape was dealt with in the introduction and entry section (see guidance in the heading above), to avoid duplication of information regarding the relevant pathways.
2.2. How important is the expected spread of this organism within the risk assessment area by human	**minimal**	**low**	There is no evidence of spread by human assistance in the risk assessment area, with the exception of

assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.			intentional releases or escapes from captive bred populations, which however pertains to the mechanism of entry (hence this is discussed in the relative section). For example, a new Sicilian population of this species was recently described by Faraone et al. (2017) according to whom the hypothesis of natural expansion along the river basin is doubtful, while the occurrence of a man-mediated introduction event is plausible (although it is not clear whether it could originate from individuals caught in the wild or from labs). Therefore this is to be considered in the context of new entries. On this regard, Lobos & Jaksic (2005) pointed out that all calculations of spread rate should be taken with caution given the possibility that there have been additional translocations by humans. No information was found on the potential transport of *X. laevis* adults and tadpoles with fish lots, as documented for other amphibian species (e.g. the American bullfrog *Lithobates catesbeianus*)
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	1) Corridors (Interconnected waterways / basins / seas). 2) “Unaided (Natural dispersal across borders of invasive alien species that have been introduced)”.		The following pathway is involved in the spread of the species: 1. Corridors (Interconnected waterways / basins / seas). This pathway fully overlaps with “Unaided (Natural dispersal across borders of invasive alien species that have been introduced)”. The main difference between the two pathways is that in the first one the species will move through the man-made infrastructures occurring in the area (i.e. interconnected waterway corridors such as channels, ditches, etc.) serving as Corridors with its own

			capabilities. Otherwise, in the Unaided category, the species is expected to move without any support from either humans or infrastructures. For example, the species is also able to spread through overland movements (see details on point 2.1 above) which by the way are intrinsically part of the movements through the waterway corridors. For this reason both pathways have been covered in the risk assessment under one single heading (Corridors (Interconnected waterways / basins / seas). The likelihood of spread in the Union based on these pathways is very high, since the likelihood of survival and reaching a suitable habitat is also very high, as documented above. There is evidence of mass overland movements of animals, estimated to number several thousand (e.g. when water bodies dry-out), and that may be driven, at least in part, by the existence of populations with high densities (Measey 2016).
Pathway name:	**[Corridors (Interconnected waterways / basins / seas)]** including **[Unaided (Natural dispersal across borders of invasive alien species that have been introduced)].**		
2.3a. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	**unintentional**	**high**	
2.4a. 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?	**likely**	**high**	As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and rivers as migration corridors. There are no specific studies providing an indication of the propagule pressure, but the species is known to

			have used this pathway successfully in the risk assessment area. However, the likelihood of this pathway to contribute effectively to the species spread is also related to the overall suitability of the area colonised. In the UK for example, small-scale migration was recorded but overall *X. laevis* did not show any evidence of dispersal into apparently favourable ponds connected by drainage channels in adjacent farmland. Furthermore, limited migration ability under typical environmental conditions was recorded within the potential overland migration range in Africa and California (Tinsley et al. 2015a). De Villiers and Measey (2017) tested also the idea of migratory movements but found no evidence. According to Tinsley et al. (2015a) the low temperature regime may have some effects on dispersal behaviour, but recent studies show that *X. leavis* is able to move even in quite cold weather conditions, hence this clearly does not prevent the species invasion (Eggert & Fouquet 2006). There is no evidence of reinvasion after eradication, but of course this cannot be excluded given the species' ability to spread undetected. For details see comments in point 2.1. above.
2.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**likely**	**high**	The species is known for having used this pathway successfully in the risk assessment area, hence the likelihood of survival is probably high if the habitat is suitable. If the interconnected waterways (such as irrigation canals, ponds, and rivers) used as migration corridors

			coincide with suitable habitats (e.g. lack of predatory fish, etc.) it is possible that the species may reproduce successfully along the pathway.
2.6a. How likely is the organism to survive existing management practices during spread?	**likely**	**medium**	No relevant management practices exist which may prevent the natural spread of the species in Europe. On the contrary, there may be practices that may favour the spread of the species. For example in Chile, the common practice of emptying dams once a year (to extract silt) may aggravate the situation by forcing the animals to migrate off periodically (Lobos & Jaksic (2005).
2.7a. How likely is the organism to spread in the risk assessment area undetected?	**very likely**	**high**	The detection of single individuals or even new populations can be difficult, given the aquatic and elusive nature of the species. Several introduced populations of *X. laevis* have gone undetected for long time periods, 2–25 years in some cases (Measey et al. 2012). van Sittert and Measey (2016) estimated that invasion debt rates - lag between the export of African clawed frogs and a rise in invasive populations - were around 15 years.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	**very likely**	**high**	According to Measey et al. (2012) irrigation channels and streams or rivers appear to be the major routes for dispersal for many invasions When these run close to artificial dams or ponds, large populations quickly become established.
2.9a. Estimate the overall potential for spread within the Union based on this pathway?	**likely**	**high**	As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and rivers as migration corridors. Spread may depend on the presence of canals. For example in Chile, a rapidly expanding viniculture industry has been assumed to have the potential to aid

			the spread of this invader, through extensive irrigation corridors, into new and previously uncolonized areas (Lobos et al. 2013). However, the likelihood of this pathway to contribute to the species spread is also related to the overall suitability of the area colonised. In the UK for example, *X. laevis* has been unable to spread far by natural means, despite being established at a small number of sites in the UK for several decades. However, it must be considered that animals occurred in a very particular system, and spread out of this area would have been very difficult (John Measey, pers. comm. 2018). Habitat connectivity is poor in the UK and, in any event, it is rarely simultaneously warm and rainy enough to encourage long distance overland movements by this species (NNSS 2011). Sousa et al. (2018) did speculate that artificial lakes of a golf course built between two sites of occurrence of the species in Portugal may have facilitated the dispersal of the species, although this is not confirmed by any definitive evidence.

End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	**very difficult**	**low**	Effective containment measures to prevent the spread of *X. laevis* through the pathway above are the same as those to control/eradicate the species, hence their applicability is clearly context dependent, and depends on the size of the population and the invasion stage.
2.11. Estimate the overall potential for spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	**rapidly**	**medium**	According to the studies carried out by Measey et al. (2012) and Ihlow et al. (2016) suitable areas (plus some limited optimal areas) fall within the Mediterranean and Atlantic biogeographic regions as well as the

			Continental and Alpine regions. Established populations are already present in the former, but not in the latter.
2.12. Estimate the overall potential for spread in relevant biogeographical regions in foreseeable climate change conditions	**rapidly**	**medium**	Further warming of the climate due to climate change may benefit the species in colonising new areas through natural dispersal. For example, by the 2070s, climate change is predicted to increase suitability in the risk assessment area, although the maps shown in the paper by Ihlow et al. (2016) do not allow for a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions may become suitable for the species.

MAGNITUDE OF IMPACT			
Important instructions: • Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**
Biodiversity and ecosystem impacts			
2.13. How important is 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?	**moderate**	**medium**	*X. laevis* is as a generalist predator able to modify its diet according to available resources (Courant et al. 2017a). Evidence exists of the negative impact on local populations of amphibians, fish, and invertebrates (Measey et al. 2012). In fact *X. laevis* is known to predate on and compete with native amphibians, including eggs and larvae (Measey et al 2015), and is thought to be a cause of trophic cascades by the consumption of benthic macroinvertebrates (Measey, 1998a; Lobos & Measey, 2002). In contrast, *Xenopus* tadpoles are primarily phytoplankton feeders (Schramm 1986). In particular, in its native range, competition and predation toward other pipid frogs (not present in the EU) was reported - i.e. on the IUCN Endangered Cape platanna (*X. gilli)* - along with predation on other anurans - i.e. the common Cape River Frog (*Amietia fuscigula)*, the clicking stream frog (*Strongylopus grayii* and the Southern Dainty Frog (*Cacosternum australis*)

			suggesting a high proportion of anurophagy, of either eggs, tadpole or adults (Vogt et al. 2017). In central Chile, *X. laevis* preys on essentially three major food types: insects, molluscs and crustaceans, while the only vertebrates found in local diets are *Xenopus* larvae (Lobos & Jaksic 2005). Indeed, predation on amphibians (including on *X. laevis* itself) represented the most frequent vertebrate taxon in several studies on the species diet (Measey et al., 2016). Another study by Vogt et al. (2017) found *X. laevis* to consume large quantities of anuran eggs and larvae. Lastly, fish, like the endangered tidewater goby *(Eucyclogobius newberryi)*, mosquito fish *(Gambusia affinis)*, and Arroyo Chubs (*Gila orcuttii)*, were found in the gut contents of *X. laevis* inhabiting the estuary of the Santa Clara River, in California (Lafferty & Page, 1997). Given the lack of evidence of long-term irreversible ecosystem change, the impact was considered moderate (see Annex II and remark in point A3 above).
2.14. 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)?	**moderate**	**medium**	Overall, the species impact on the risk assessment area is similar to the impact described in regions beyond the EU. For example, according to Measey (1998) *X. laevis* in South Wales ate a wide variety and size range of prey. Zoobenthos and zooplankton made the largest contribution to diets, followed by terrestrial invertebrates. Vertebrate preys (other than eggs and larvae of the same species) were also present, i.e. a bank vole (*Clethrionomys glareolus*) eaten alive or recently dead, and a chick (unidentified) probably eaten as a carrion. In fact, it is important to consider that *X. laevis*

			is also able to detect and feed on carrion (Measey 1998). Amaral & Rebelo (2012) confirmed the predation by *X. laevis* on eggs and adults of native amphibians, as well as on native fish in Portugal. The diet included benthic preys, with water snails (Physidae) being the most important, followed by the invasive American crayfish (*Procambarus clarkii*), but also native amphibians (including *Rana perezi* skeletons and egg masses) and fish (among which *Cobitis paludica*, a vulnerable Iberian endemic). In France, Grosselet et al. (2006) speculated that *X. laevis* may predate on eggs of large newts (i.e. *Triturus cristatus* and *Triturus marmoratus*). Also Courant et al. (2018a) showed that species richness of native amphibians was negatively related to the abundance of *X. laevis*, despite some methodological bias discussed by the authors themselves. In particular, in France a significant decrease in the proportion of nektonic macroinvertebrates was reported in ponds occupied by *X. laevis* (Courant et al. 2018b). A study by Faraone et al. (2008) in Sicily shows that the most important prey categories are nektonic and planktonic organisms, and confirmed the presence of *X. laevis* eggs and larvae as well as terrestrial invertebrates (odonates and mayflies) in the diet. Additionally, Lillo et al. (2010) showed that presence of *X. laevis* in Sicily is associated with a decline in the reproduction of native amphibians (namely *Discoglossus pictus*, *Hyla intermedia* and *Pelophylax synklepton esculentus*). However, no one native amphibian was present in the diet of the species. Only conspecific tadpoles were found, confirming the significant cannibalistic behaviour

			of this species. The study by Lillo et al. (2010) also shows that the almost total absence of overlap of the trophic niche suggests the lack of competition for trophic resources between the alien species and the native ones.
2.15. 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?	**moderate**	**low**	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 one, it is likely that the level of risk will at least be confirmed as "Moderate" also in the future.
2.16. 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?	**moderate**	**low**	In the light of the suggested impact on the amphibian species occurring in Italy and France and protected by the Habitats directive, the decline in conservation value caused by *X. laevis* is considered as "Moderate".
2.17. 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?	**moderate**	**low**	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 causing the extinction of any native species, it is likely that the level of risk will at least be "Moderate" in the future.
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	**moderate**	**high**	The impact of *X. laevis* on ecosystem services is caused by predation with possible accumulative effects in the ecosystem, including increased competition with other species for food (see point 2.13 and 2.14) and its functioning as a pathogen vector (see point 2.28). *X. laevis* might also have indirect impacts on the aquatic system such as increasing water turbidity and nutrient release caused by *X. laevis* disturbing the sediment (Lobos and Measey 2002).

			Consequently, *X. laevis* has been reported to negatively affect the invaded ecosystems, and as a consequence has been ranked as having the second greatest impact on native ecosystems by any amphibian (Measey et al., 2016). See also the assessments by Kumschick et al. (2017a), Kumschick et al. (2017b) and Kraus (2015) already discussed in point A3 of this document.
2.19. 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)?	**moderate**	**high**	Overall, the species impact in the risk assessment area is likely to be similar to the impact in regions beyond the EU, as described above, namely on: 1) Provisioning (Biomass: Reared aquatic animals); 2) Regulation & Maintenance (Regulation of physical, chemical, biological conditions: Lifecycle maintenance, habitat and gene pool protection, Pest and disease control, Water conditions).
2.20. 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?	**moderate**	**low**	There is no documented evidence of the species being able to cause other types of impact, hence the level of risk can be expected to be "moderate" in the future.
Economic impacts			
2.21. 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	**minor**	**low**	Due to increased predation and/or competition for food, *X. laevis* is known to interfere with aquaculture, leading to possible economic costs. While no quantitative estimates about the economic impacts are available, in South Africa, for example, *X. laevis* is considered a threat to fresh-water aquaculture of common carp (*Cyprinus carpio*) and Chinese silver carp (*Hypophthalmichthys molitrix*) mostly because of competition for food (Schramm 1987). Additionally, it is considered a constraint on the production of the giant freshwater prawn (*Macrobrachium rosenbergii*), mainly

			due to predation (Taylor et al., 1992). Outside its native range, in Japan, the African clawed frog was found to have an impact on aquaculture by preying on juvenile carp (Kokuryo, 2009). In particular, a study by Schramm (1987) in South African aquaculture ponds, revealed that farmed fish larvae constituted a large proportion of *X. laevis* stomach contents (up to 25%), and that small fish <1 g are particularly vulnerable (although it does not necessarily represent the typical diet of native populations, see Courant et al. 2017a). Furthermore, in a study by Schramm (1987), it seemed likely that competition with *X. laevis* tadpoles was at least partly responsible for the slower growth of *H. molitrix*. In addition to the above, a reported problem in South Africa concerns the mass migrations leading to large numbers of clawed frogs invading houses and clogging up irrigation pipes (Somma 2018, Tinsley et al., 1996), but also in this case no figure is available. Following the SEICAT scheme developed by Bacher et al. (2018), the impact category for this species should therefore fall in between Minimal concern and Minor. No information/data is available on the costs for management, despite the many management activities carried out on the species. The only exception is an estimation of the man days reported for the control of the species in a pond in South Africa (De Villiers et al 2016). According to the authors, 27 person days for 338 *X. laevis* from one impoundment were needed, while regular seining could require as little as eight person days per year. As a side not, according to John Measey, pers. (comm. 2018) the impoundment size is 603 m2 (see also Vogt et al 2017).

			Lastly, a LIFE project aimed at the control of *Xenopus laevis* - together with the American bullfrog (*Lithobates catesbeianus*) - is currently in progress in France: LIFE15 NAT/FR/000864 LIFE CROAA - Control stRategies Of Alien invasive Amphibians in France (for details, see https://www.life-croaa.eu). The project, co-funded by the EU through the LIFE program, has a total budget of 3,430,179.00 € (see http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=5842). However, as this also targets species other than *X. leavis* are targeted, and since the project is still in progress, it is not possible to have clear figures on removal costs for *X. laevis* in particular.
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	**minimal**	**low**	No information/data is available on the economic costs caused by *X. laevis*. In the UK the economic losses caused by this species, if any, were considered likely to be minimal given the limited distribution and very small numbers of *X. laevis* that were present (NNSS 2011).
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	**minimal**	**low**	In case of a future expansion of the species range, some economic impact and associated costs may be evidenced, e.g. on aquaculture activities or other sectors. While there is no documented evidence of the species causing this type of impact in the risk assessment area, it is not possible to exclude that this could happen in the future. However, for the time being it should be considered minimal.
2.24. 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)?	**moderate**	**high**	The only figures that are available in the risk assessment area concern the activities carried out in France through the project LIFE CROAA, Control stRategies Of Alien

			invasive Amphibians in France (LIFE15 NAT/FR/000864). This project aims to limit the expansion of *X. laevis* along dispersal corridors (together with the eradication/control of *Lithobates catesbeianus* in several sites). The total budget of this project is of 3.43 million euro and it will be carried out in the period 2016-2022 by the Société Herpétologique de France and other partners.
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	**major**	**low**	In case of a future expansion of the species range in the risk assessment area, the economic costs / losses associated with managing *X. laevis* may rise accordingly.
Social and human health impacts			
2.26. 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).	**minimal**	**low**	No information has been found on the issue.
2.27. 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.	**minimal**	**low**	No information has been found on the issue
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	**minor**	**low**	The most serious impact usually attributed to *X. laevis* is related to its potential role in the introduction and spread of the chytrid fungus, *Batrachochytrium dendrobatidis (Bd)*, the cause of amphibian deaths and population declines in several parts of the world (Weldon et al. 2004). Bd disease has been implicated in mass mortalities and widespread declines in European amphibian species, like common midwife toad (*Alytes obstetricans*) (Bosch et al., 2001) and fire salamander (*Salamandra salamandra*) (Bosch & MartínezSolano,

			2006) in Spain. However, to date there is no evidence that *X. laevis* has functioned in this role of Bd vector or has caused impact on native amphibians through this mechanism. For this reason, the impact of the species was considered “minor” by Kumschick et al. (2017b, see in particular the supporting information annexed to the relevant paper). As a remark, the same authors discussed a previous assessment by Kraus (2015) based on the assumption that *X. laevis* contributed to the spread of Bd which then caused declines in native species, but which is not demonstrated (De Busschere et al. 2016, John Measey, pers. comm. 2018). Hence a higher score would not be justified. *Xenopus laevis* was also identified as a potential vector of ranavirus (Robert et al., 2007). Although a causal link between *X. laevis* and the dispersal of these pathogens is not demonstrated (Measey et al. 2012), this frog could play a role in the spread of disease, by acting as an asymptomatical reservoir/vector for both diseases, without being susceptible or just suffering sublethal effects. This seems to be confirmed at least for the chytrid fungus by studies on either wild or captive animals in the UK, Chile, and USA (Tinsley et al. 2015b, Solís et al. 2010, Soto-Azat et al. 2016, Vredenburg et al. 2013), but not in France (Ouelletet al. 2012). Additionally, *X. laevis* may carry several other parasites and pathogens, like *Chlamydia* (Howerth et al. 1984, Reed et al. 2000) and many others, in both its native range and the alien range (Kuperman et al 2004, Tinsley, 1996). For example, according to Lafferty & Page (1997), three internal parasites were observed in or on

			the gut (although a complete parasitological assessment was not undertaken). The African tapeworm *Cephalochlamys namaquensis* was found in intensities from 6-25 individuals (including several mature adults) in the anterior duodenum. It was not previously reported outside of Africa, hence it may have entered other areas with this species. Ciliates of the genus *Nyctotherus* (0.25 mm trophs) were present in abundance posterior to the section of the gut where tapeworms occurred. Larval nematodes were encysted on the outside of the stomach wall (might be transferred to the birds that eat them, potentially leading to some pathology).
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	**NA**		No information has been found on the issue
2.30. 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?	**major**	**low**	The natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area, is not expected to mitigate the impact of *X. laevis* in relation to its role as a vector of dangerous parasites and pathogens to the native fauna.

ANNEXES

ANNEX I	Scoring of Likelihoods of Events
ANNEX II	Scoring of Magnitude of Impacts
ANNEX III	Scoring of Confidence Levels
ANNEX IV	Ecosystem services classification (CICES V5.1) and examples
ANNEX V	Biogeographic Regions and MSFD Subregions
ANNEX VI	Template for Annex with evidence on measures and their implementation cost and cost-effectiveness
ANNEX VII	Species distribution model NB: work in progress

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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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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 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.

[10] Not to be confused with „no impact".

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.*. 2017)

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[11]	**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.*

[11] 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.

	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** 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

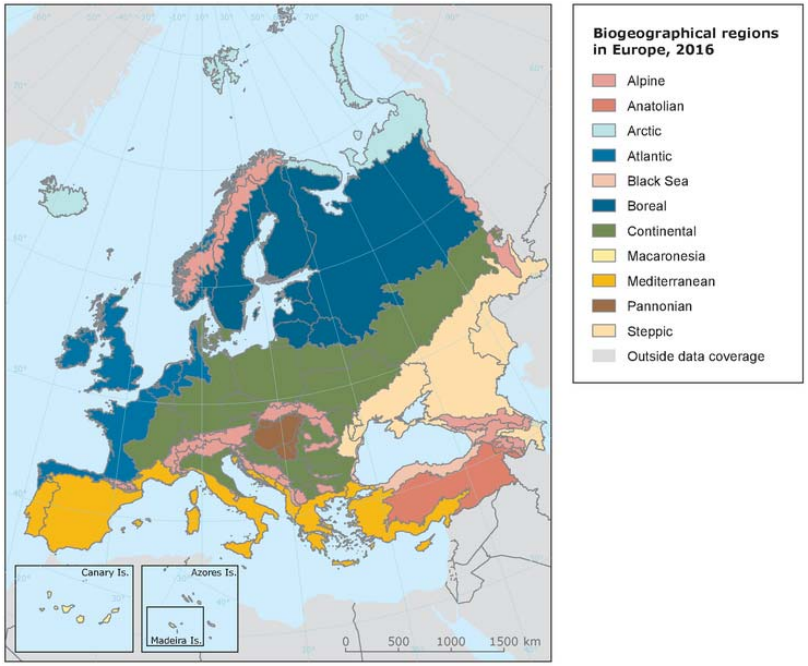
			Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific 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.*

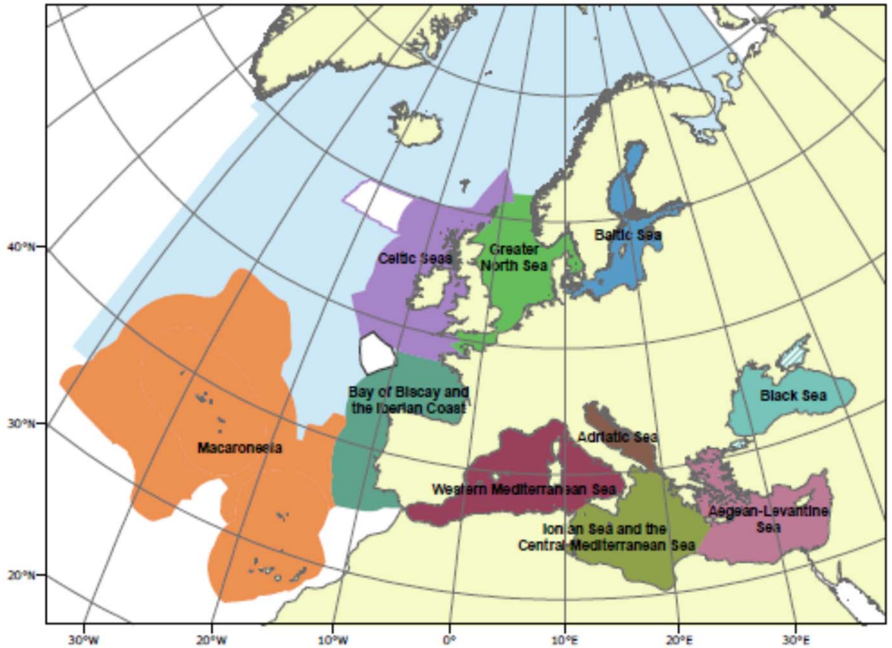
ANNEX V EU Biogeographic Regions and MSFD Subregions

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 VII Species distribution model

Projected distributions were obtained from the authors of two existing studies reporting three global-scale species distribution models for *Xenopus laevis* (Measey et al 2012; Ihlow et al 2016). Both studies used a similar distribution database, with more record cleaning in the later paper, and a similar set of input variables and modelling methods (Table 1). However, the three models varied considerably in which climate variables were used to predict suitability (Table 1), suggesting high uncertainty in using the outputs of the models for this assessment.

The authors of both papers supplied shapefiles with the projected suitable regions, revealing marked differences in the European regions predicted to be suitable in the current climate (Figure 1). All models predict substantial suitable regions in Portugal, Spain, France and Italy. The models of Ihlow et al (2016) also predict large suitable regions in eastern Europe, based on a minimum training presence threshold. This threshold probably overestimates the suitable region in Europe as the species has been introduced and recorded in marginal conditions in northern Europe and suitability gradient maps shown in the paper suggest moderate to high suitability only in warm western Mediterranean regions. The predictions from the Measey et al (2012) model should be treated with caution as the Maxent model is less reliable than the ensemble model (John Measey pers. comm. 2018). Also, this study did not consider suitability under different emission scenarios (Table 1).

Climate change projections supplied for the 2070s from Ihlow et al (2016) differed markedly between emissions scenarios and the Maxent and Ensemble models. However, these projections do not appear consistent across scenarios (e.g. RCP4.5 should be intermediate between RCP2.6 and RCP6.0, but it is not in all cases) and were probably influenced by an overly liberal minimum training threshold choice.

Overall, the information supplied was considered too uncertain to usefully identify suitable regions beyond the currently invaded regions of Europe.

Table 1. Comparison of published species distribution models for *Xenopus laevis*.

	Measey et al (2012)	**Ihlow et al 2016 (Maxent)**	**Ihlow et al 2016 (Ensemble)**
Number of native range records	1075	826	826
Number of non-native range records	124	99	99
Spatial resolution	2.5 arcminutes	2.5 arcminutes	2.5 arcminutes
Predictor variables from Worldclim	Isothermality (bio3) Minimum temperature of the coldest month (bio6) Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Precipitation seasonality (bio15) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of coldest quarter (bio19)	Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Mean temperature of the coldest quarter (bio11) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of the warmest quarter (bio18) Precipitation of coldest quarter (bio19)	Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Mean temperature of the coldest quarter (bio11) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of the warmest quarter (bio18) Precipitation of coldest quarter (bio19)
Modelling software	Maxent	Maxent	Biomod
Background definition	Radius of 250 km around the records	Radius of 250 km around the records	Radius of 250 km around the records

	Measey et al (2012)	**Ihlow et al 2016 (Maxent)**	**Ihlow et al 2016 (Ensemble)**
Reported predictor importance	Isothermality (27.4%) Minimum temperature of coldest month (19.8%) Precipitation of coldest quarter (11.7%) Mean temperature of warmest quarter (10.4%) Mean temperature of wettest quarter (8.8%) Temperature annual range (6.7%) Precipitation of wettest quarter (6.6%)	Precipitation of driest quarter (27.7%) Mean temperature of wettest quarter (16.8%) Mean temperature of coldest quarter (14.5%) Precipitation of warmest quarter (11.4%) Precipitation of coldest quarter (8.3%) Temperature annual range (7.0%) Mean temperature of driest quarter (6.2%) Precipitation of wettest quarter (6.2%) Mean temperature of warmest quarter (1.9%)	Mean temperature of coldest quarter (19.1%) Precipitation of warmest quarter (16.6%) Mean temperature of warmest quarter (13.9%) Precipitation of driest quarter (12.6%) Precipitation of coldest quarter (8.3%) Mean temperature of driest quarter (8.3%) Precipitation of wettest quarter (8.0%) Mean temperature of wettest quarter (7.6%) Temperature annual range (5.0%)
Threshold(s) to project suitable region	Minimum training presence and 10% training omission	Minimum training presence	Minimum training presence
Masking to prevent extrapolation	Multivariate Environmental Similarity Surface (MESS)	Multivariate Environmental Similarity Surface (MESS)	Variable clamping
Climate change scenarios	None	2070s under RCP2.6, RCP4.5, RCP6.0 and RCP8.5	2070s under RCP2.6, RCP4.5, RCP6.0 and RCP8.5

Figure 1. Projected European regions suitable for establishment by *Xenopus laevis* from three modelling approaches. In (a) the suitable region is defined using two thresholds, with almost no parts of Europe projected suitable under the stricter 10% omission threshold. The threshold used in (b) and (c) is the minimum training presence, and suitable areas are shaded red. In all plots, regions where extrapolation prevented prediction are shown in black.

(a) Measey et al (2012) Maxent model (b) Ihlow et al (2016) Maxent model (c) Ihlow et al (2016) Ensemble model

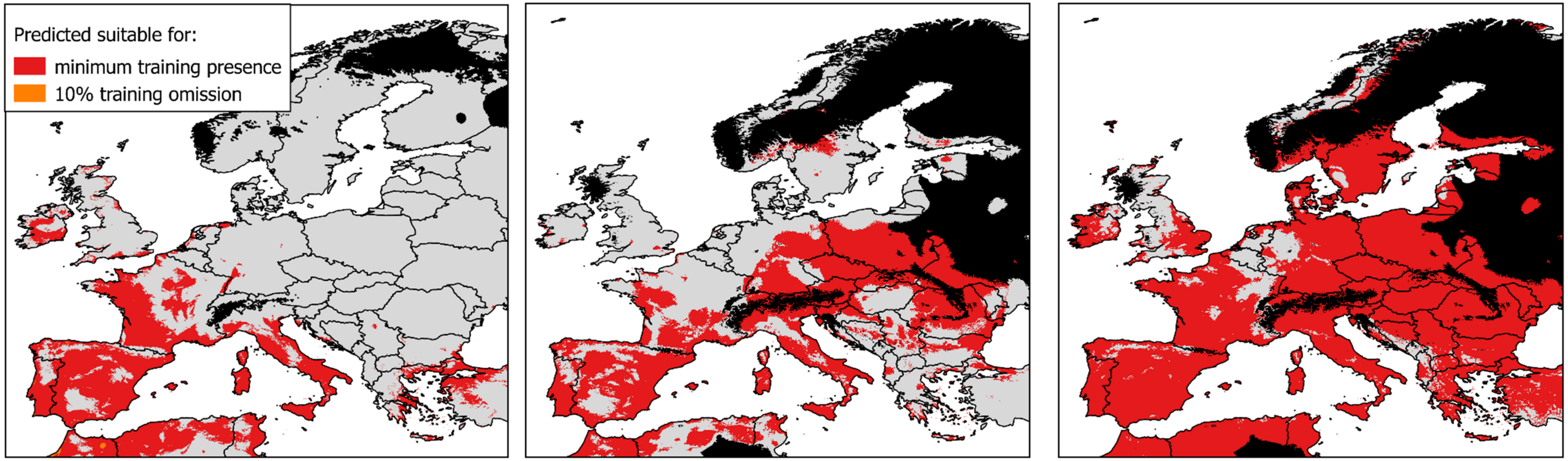
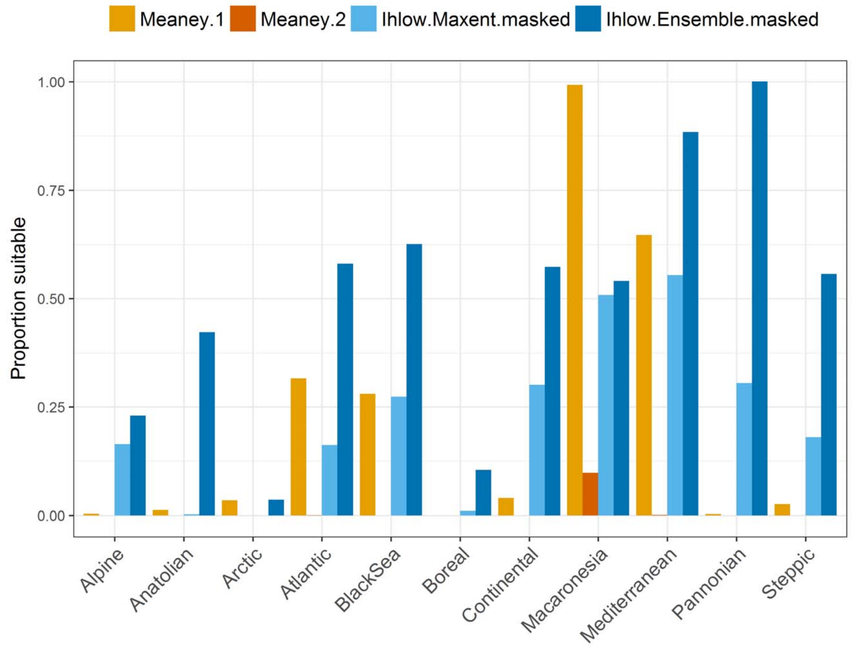


Figure 2. Variation in projected suitability among Biogeographical regions of Europe (Bundesamt fur Naturschutz (BfN), 2003) from the different model outputs supplied. The regions are shown in the right hand map. Measey.1 and Measey.2 differ based on thresholding by the minimum training presence or a stricter 10% omission rate, respectively.



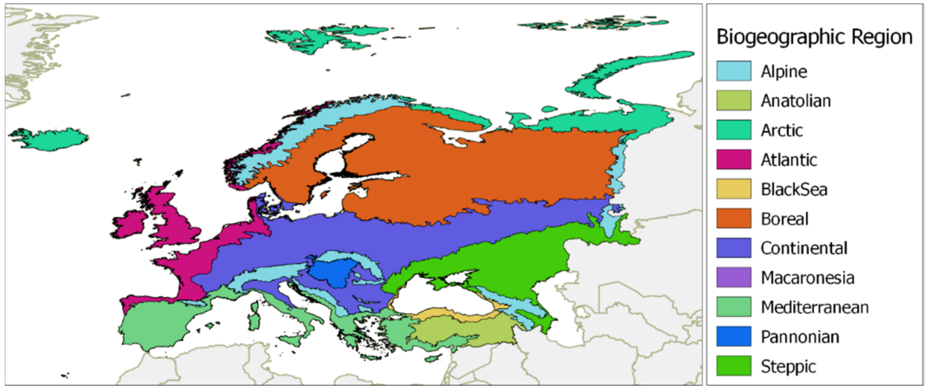
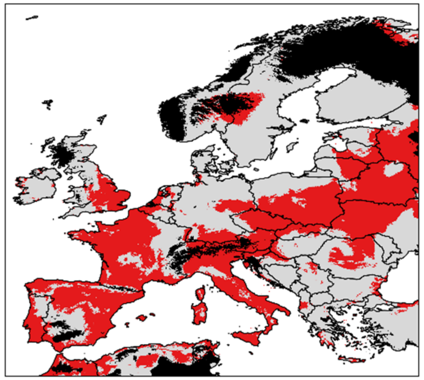
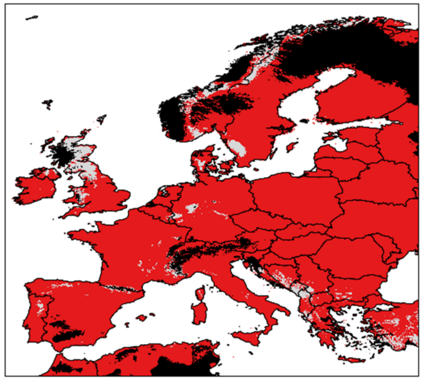


Figure 3. Projected European regions suitable for establishment by *Xenopus laevis* in 2070 under four emissions scenarios. Suitable areas above the suitability of the minimum training presence are shaded red. Regions where extrapolation prevented prediction are shown in black.

(a) Ihlow et al (2016) Maxent – RCP2.6

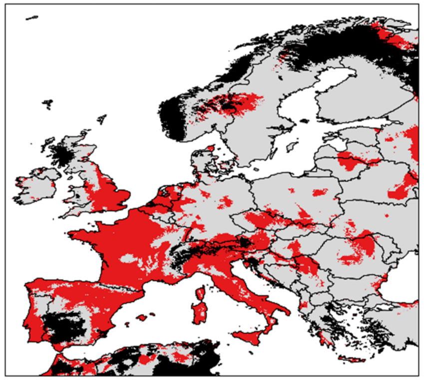
(b) Ihlow et al (2016) Ensemble – RCP2.6

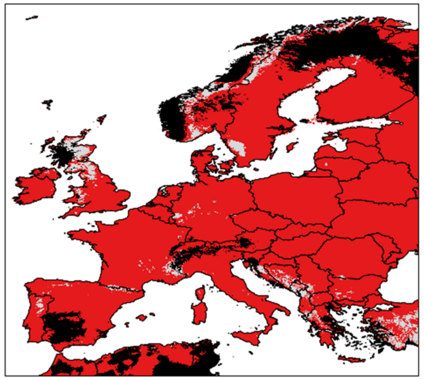




(c) Ihlow et al (2016) Maxent – RCP4.5

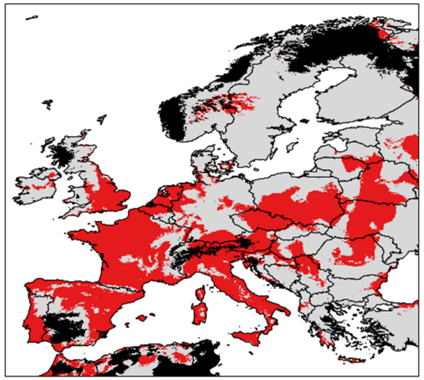
(d) Ihlow et al (2016) Ensemble – RCP4.5

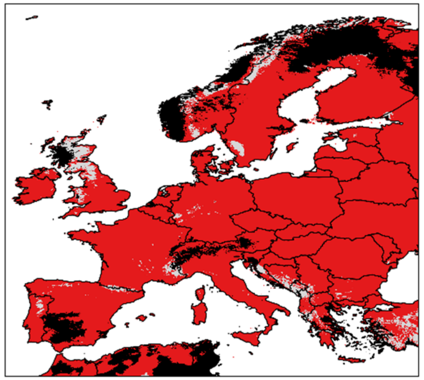




(e) Ihlow et al (2016) Maxent – RCP6.0

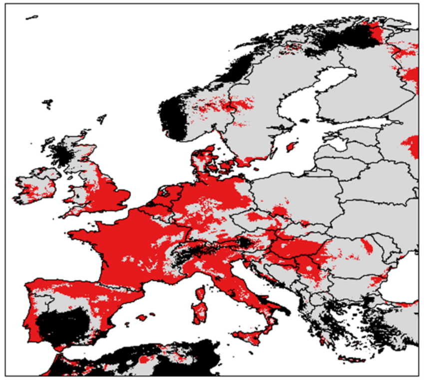
(f) Ihlow et al (2016) Ensemble – RCP6.0

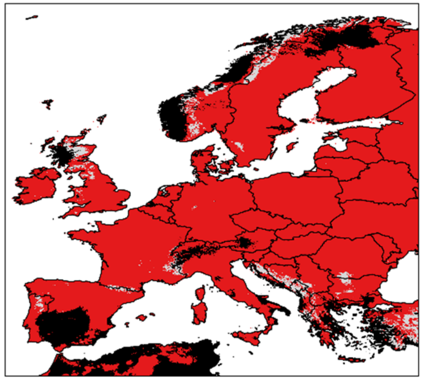




(g) Ihlow et al (2016) Maxent – RCP8.0

(h) Ihlow et al (2016) Ensemble – RCP8.0





Template for Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	*Xenopus laevis*
Species (common name)	African Clawed Frog
Author(s)	Pete Robertson, Riccardo Scalera
Date Completed	22/09/2018

Summary
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.

Methods to achieve prevention
This species is widely kept and traded through the pet trade and is used as a laboratory species. Established populations in Europe are thought to have established through the accidental or deliberate release of captive animals. The adoption and enforcement of appropriate legislation (Art. 7 of the Regulation (EU) 1143/2014) and codes of best practice targeted to commercial and non-commercial owners in Europe to reduce the risks posed by these pathways should reduce the probability of further introductions. Raising awareness of the problems posed by the release or presence of this species, and invasive species in general, should reduce the risk of further escapes and the rapid reporting of new populations to support a rapid response.

Methods to achieve eradication Small eradication campaigns have been carried out successfully in the UK, Spain, and the USA (Measey et al. 2012) but these have only been possible in small areas and at an early stage of invasion. The chances of success are strongly influenced by the characteristics of the waterbodies affected (e.g. type, size, and overall network). A combination of methods, including traps, fyke-nets, hand-dipping and electro-fishing are favoured. For a limited range of sites, pond-draining, destruction or the addition of salt to the waterbody may be appropriate. The release of sterile males may be effective in future but further development of its use on amphibians is required.

Methods to achieve management
These methods may also play a role in the long-term management of the species, although the prospects for effective management will be site specific. Fencing is already used to limit the movements and dispersal of amphibians and may play a role in limiting dispersal or protecting sites in specific circumstances

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	**Managing pathways** *X laevis* is widely held within the European pet trade and used as a research model in laboratories. The accidental and deliberate releases of these animals is thought to have been the primary pathways of introduction to Europe (Measey et al. 2012, Tinsley et al. 2015). The adoption and enforcement of appropriate legislation (Art. 7 of the Regulation (EU) 1143/2014) and codes of best practice targeted to commercial and non-commercial owners in Europe to reduce the risks posed by these pathways should reduce the probability of further introductions.	To prevent escapes of the African clawed frog, Ihlow and co-workers (2016) stressed the importance of biosecurity at breeding facilities.	
	Effective reporting of new incursions. *X.laevis* is a distinctive species that is not likely to be mistaken for other native anurans in Europe. However, the complex classification of this and closely related species may lead to problems identifying new sightings. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the species can become established.		
	Raising awareness Raising public awareness of the risks posed by invasive alien species in general, and anurans in particular, with examples on the impacts. This can include the production of targeted publicity and identification material, and the involvement of the general public, along with key stakeholders, in citizen science initiatives. Example publicity and identification material includes		

	https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=86494&inline		
	Preparation of Contingency Plans Rapid response to new incursions is likely to be the best approach to prevent the wider establishment of this species. They have the ability to spread rapidly between water bodies, the estimated annual spread of feral populations varied between 1 km in France (Fouquet and Measey 2006) and 5.4 km in Chile (Lobos & Jaksic 2005), and the feasibility of eradication is likely to drop dramatically as the species spreads. The preparation of contingency plans, including the organisation to be responsible, staffing, equipment and sources of funding, should be prepared in advance to support any rapid response		
Methods to achieve eradication	**Aquatic funnel traps and fyke nets**	Such methods do now allow the removal of all individuals of a population. Therefore, they are useful only if the objective is the control of the species (hence the reduction of population densities), and not the eradication of the species (John Measey, pers. comm. 2018). The most widespread method used to catch this species, both for study and to support control, is the use of aquatic traps. Tinlsey & McCoid (1996) describe a successful eradication in Virginia, USA, using aquatic trapping, aided by severe weather. There is good evidence of success with large funnel traps constructed from buckets or bins with conical funnels inserted in the side. (e.g. Lobos & Measey, 2002, Rebelo et al 2011). Modified fyke nets have also been used effectively (Measey & Tinsley, 1998). Also Lafferty and Page (1997) suggested that the use of traps may be the best option to lower densities of the African clawed frog in California.	High. These methods have been used to support successful eradications of this species from small water bodies although they will need to be used in association with other methods.

		This species locates its prey by olfactory means, and they can be attracted over significant distances to appropriately baited traps. Liver and catfood have both been used as effective baits. According to Tinsley et al. (2015) the use of baited traps for live capture of *X. laevis* is highly effective. However, the use of traps, may be not sufficient to remove all individuals from a pond, as it would take too long and doesn't stop animals moving between sites (John Measey, pers. comm. 2018). For example after 3 years trapping in the UK a marking study using these methods was still catching new unmarked animals (Measey 2001). Trapping carries the risk of non-target captures, and may impact on the welfare of any animals caught. The regular checking of traps to reduce the time animals may be held, and the release of non-targets can reduce these risks.	
	Hand, dip-net and seine net	Hand capture and dip-netting can be used to reduce the number of individuals from a water body, hence while they would not be suitable for eradications, may be considered as control methods (John Measey, pers. comm. 2018). For example, they were used to remove larvae (Rebelo et al 2011, Vale, 2010, Sousa et al. 2017, Sousa et al. 2018). Although this approach has been used also to remove the egg masses of other amphibians, egg masses are not laid by this species and eggs are deposited singly or max 2-3 onto substrates (John Measey, pers. comm. 2018). Additionally, these methods are not effective for adults and juveniles unless the pond is very small. Seine netting has been used, apparently with some success, to remove adults and juveniles as part of an invasive species control project in South Africa (Measey & Davies, 2011).	High. This method has been used to assist with the control of this species, although its effectiveness will be limited.

		In Portugal, as reported by Sousa et al. (2018) a traditional fishing technique named xávega was applied in some artificial habitats, like irrigation and ornamental ponds: it consists of dragging a vertically-maintained fishing net along the bottom of the pond—to capture the tadpole swarm. This approach is highly selective and unlikely to raise public concern. However, the effect that reducing densities of tadpoles has on survival is not known, and in many species, this has a counter effect (John Measey, pers. comm. 2018).	
	Pond draining	Such methods do now allow the removal of all individuals of a population. Therefore, they are useful only if the objective is the control of the species (hence the reduction of population densities), and not the eradication of the species (John Measey, pers. comm. 2018). Pond draining (combined with capture) has been used to successfully eradicate populations in North Carolina, USA, apparently aided by freezing weather (Tinsley & McCoid, 1996), and at another site in California, USA (Measey et al, 2012). It has also been effective at a site in Spain (Pascual et al, 2007). Draining assists the capture of remaining animals, though care must be taken to avoid triggering overland dispersal; hence, fencing around the pond is a sensible precaution. This method is only likely to be practical for use on small waterbodies although it is likely to be effective. It will also impact on other species using the site and could raise public concern. In Portugal it has been used successfully in a lake of a golf course, in association to other methods, including electrofishing, netting and use of chemicals, i.e. sodium hypochlorite (Sousa et al. 2017).	Medium. These methods have been used to support successful eradications of this species from small water bodies, particularly when used alongside other methods, but the actual efficacy of the method is poorly documented, as most evidence is anecdotal (John Measey, pers. comm. 2018).

		However, animals are able to survive in mud, and so may not be captured even when a pond is drained (John Measey, pers. comm. 2018).	
	Pond destruction	In some cases it may be practical to destroy the breeding pond, for example by draining and filling it in. This would need to be accompanied by fencing to prevent the dispersal of the species. This would only be applicable in specific circumstances where the loss of the water body and corresponding non-target impacts was considered acceptable. However, this approach has been used on occasion to remove severe infestations with invasive aquatic weeds. However, given that individuals are likely to survive being buried, this method may lead to the overland dispersal of surviving animals (John Measey, pers. comm. 2018) although this could be mitigated by the use of fencing.).	Medium. This approach has been used to manage other invasive aquatic species although not documented for use with amphibians. Its practicality and acceptability are likely to be limited to very specific circumstances
	Electro-fishing (also known as electric fishing, electro-shocking, or electro-frogging)	Electro-fishing has been used to capture adult *Xenopus* with some success in Portugal (Rebelo et al. 2011, Sousa et al. 2017, Sousa et al. 2018) coupled with other methods. In small, accessible waterbodies it may provide a practical, relatively effective and inexpensive approach (after the initial outlay for equipment). It will have some impact on non-target species, however, and must be subject to careful health & safety assessment. However, this method is unlikely to remove all animals in a pond if used in isolation (John Measey, pers. comm. 2018).	High— Known to be effective for this species, particularly when used alongside other methods, but its use is likely to be limited to small waterbodies
	Addition of fish as predators or competitors	*X. laevis* is eaten by large fish, turtles, frogs, snakes, aquatic insects, and birds (Lafferty & Page, 1997), and this species is reported to favour waters that do not contain predatory fish. (McCoid and Fritts, 1980, Tinsley et al. 2015).	Medium – Predation is well documented for this species, but eradication

		Prinsloo et al. (1981) reported the successful depletion of frog numbers following the addition of predatory largemouth bass (*Micropterus salmoides*). Chinese silver carp, *Hypophthalmichthys molitrix* or Chinese bighead carp *Aristichthys nobilis* Richardson may also be useful species to control *X. laevis*, as both species compete with *Xenopus* larvae for phytoplankton as food. However, this approach is likely to result in significant long-term changes to any water body with impacts on non-target species. It is possible that this approach would encourage animals to move overland (John Measey, pers. comm. 2018).	through the addition of predatory species has not been demonstrated and would face issues of practicality and acceptability.
	Biocides Rotenone is widely used as a piscicide (McClay 2000) and acts by interfering with cellular respiration, such that affected fish rise to the surface in an attempt to gulp air, where they are more easily caught	The addition of biocides to the water has been attempted as a control method for this species. McCoid and Bettoli (1996) document an unsuccessful use of Rotenone against this species in California. Despite high concentrations of Rotenone which were toxic for tadpoles, it was ineffective against adult frogs, possibly because adult clawed frogs are air-breathers (McCoid and Fritts 1980) Understanding of effective biocides for *X. laevis* is currently limited, and concerns over non-target effects will likely limit their application.	Medium – the use of biocides is well documented, but the use of Rotenone appears ineffective for this species.
	Chemicals (salt) As summarised by Sousa et al. (2017) In Portugal sodium hypochloritehas been usedhypochlorite while refilling with water a lake of a golf course where an eradication attempt was undertaken (. by draining the lake and removing the animals with the use of electrofishing and netting) with encouraging results.	An attempt to remove *X. laevis* in Washington State (USA) appears to have been successful using salt, drift fences and pit falls (John Measey, pers. comm. 2018). As with draining and other biocides, concerns over the non-target effects of adding salt may limit the application of this method.	Medium. Salt has been used effectively in Washington together with pit fall and drift fences. However, this account is currently only a

			personal communication, not a published method. Itis being used in Portugal in association with other methods such as draining, electrofishing and netting.
	Sterile Males. The sterile male technique (Knipling 1959) is a method of biological control, whereby overwhelming numbers of male animals are released into the wild. The sterile males compete with wild males to mate with the females. Females that mate with a sterile male produce no offspring, thus reducing the next generation's population. Sterile males are not self-replicating and, therefore, cannot become established in the environment. Repeated release of sterile males over low population densities can further reduce and in cases of isolation eliminate pest populations Sterilization in amphibians can be generated by exposing recently fertilised eggs to pressure or cold shock, producing individuals with triploid genes (Descamps and DeVocht 2017) who are sexually capable but infertile. Sterile males of *X. laevis* were produced in past experiments (Tompkins 1978), although this was not related to any attempt to use sterile males for biological control.	This technique has never been used to eradicate any amphibian, although it has potential for development. The production of large numbers of sterile males would come at a cost, and these would need to be able to successfully compete for mates with fully functional males in the wild. The competitive ability of triploid animals would need to be tested. The release of large numbers of sterile males would also have impacts on native species and habitats, increasing any impact caused by the target population. It would also require careful presentation to the public and landowners to avoid misunderstanding about the need to prevent escapes and releases, while doing the same as a control measure.	Low. This method was never used to eradicate amphibians.

	Surveillance Determining the presence, absence or abundance of the species is a key requirement for effective eradication or long-term management. This species is readily identifiable and visual searches, or the use of the methods described above as sampling tools can all inform surveillance. Generic tools for surveying amphibians (Sutherland 2006, Dodd 2010) The detection of environmental DNA provides an alternative approach to determine the presence and distribution of species, including amphibians (Muha et al 2017). For example, according to a study by Secondi et al. (2016) the method can be successfully applied to survey invasive populations of *X. laevis* even at low density in order to confirm suspected cases of introduction, delimit the expansion of a colonized range, or monitor the efficiency of a control program.		High. Environmental DNA is a proven method suitable for the survey of amphibians.
Methods to achieve management	All of the methods described above can contribute to the long-term management of this species, although their practicality and effectiveness will be heavily influenced by local site considerations		
	Fencing and barriers Fencing is widely used to restrict the movements of amphibians during development operations. A variety of designs are commercially available (search term - newt fencing). In South Africa, a concrete wall was constructed to exclude *X. laevis* and ensure the conservation of *X. gilli* (Picker and de Villiers, 1989). However, this was unsuccessful (John Measey, pers. comm. 2018),	As with other invasive amphibians, fencing could be useful in restricting dispersal of newly introduced populations (Prinsloo et al, 1981). It can also help limit movements from sites undergoing control such as draining or pond destruction or protect vulnerable sites from colonisation. It may assist with removal operations and it will reduce the chance of further colonisation. Fencing carries ongoing costs of maintenance and will also restrict the movement of other species.	Medium. The use of fences to limit amphibian movement is well described, although the failure of this method in South Africa limits the confidence in this assessment.

	Addition of fish as predators to unoccupied waterbodies to limit colonisation According to John Measey (pers. comm. 2018) fish appear to influence which waterbodies this species will colonise, and it is possible that this could be used to manage ongoing spread.	The addition of fish to unoccupied waterbodies would have consequences for other species occupying those sites. The effects on colonisation have yet to be demonstrated, and the prospects for the use of this approach will be restricted by the local distribution of alternative sites.	Low. This approach has yet to be used in practice.

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2[1]

Name of organism: *Fundulus heteroclitus* (Linnaeus, 1766)

Photo in the public domain by National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce, https://commons.wikimedia.org/w/index.php?curid=1549976. The two brightly coloured fish at top and bottom are males, with a duller female betweem them.

Author(s) 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.

[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

Peer review 1: Dr. Wolfgang Rabitsch, Environment Agency Austria, Vienna, Austria
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This risk assessment has been peer-reviewed by three independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study.

Date of completion: 23/10/2018

Contents

RISK SUMMARIES

	RESPONSE	CONFIDENCE[2]	COMMENT
Summarise Entry[3]	likely	medium	This species is very abundant in the native area (eastern coast of North America), with a long latitudinal range (from Florida to Canada) and very hardy. The species is present in the aquarium hobby and used in laboratory research. It could also be imported in contaminated bait or in ballast water, since it is among the most abundant fish species in estuaries of eastern North America. Although not widely introduced worldwide, it is thus likely to entry into the risk assessment area based on a number of pathways (ESCAPE FROM CONFINEMENT (Pet / aquarium / terrarium); ESCAPE FROM CONFINEMENT (Research & ex-situ breeding); TRANSPORT – CONTAMINANT (Contaminated bait); TRANSPORT - STOWAWAY (Ship/Boat ballast water)). The likelihood is similar in different biogeographical regions except the ones without coastal areas (e.g. Pannonian region).
Summarise Establishment[4]	very likely	high	The habitat of *F. heteroclitus* is located in brackish or saltwater, and inhabits sheltered coastal areas such as saltmarshes, tidal creeks, estuaries, or bays. This habitat is quite specific but common in Europe. *F. heteroclitus* is a very hardy species, eurythermic and euryhaline, with a wide latitudinal range in the native area. It has already established abundant populations in two distant Iberian regions and is likely able to establish in many other regions of the risk assessment area (European Union). It has been suggested to be limited by the

[2] In a scale of low / medium / high, see Annex III

[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

			existence of benthic muddy saltmarsh environments, which are only found near major estuaries or lagoons areas.
Summarise Spread[5]	slowly	high	Mummichogs are rather sedentary species, with small home ranges. They have naturally spread in the Iberian Peninsula through saline waters, but to neighbouring areas and quite slowly. Excluding intentional pathways, it could also spread within the risk assessment area through contaminated bait or ballast water.
Summarise Impact[6]	moderate	low	There is observational evidence that the mummichog is causing population declines of *Aphanius baeticus* and *Aphanius iberus*, two endangered cyprinodontid fish, endemic to Spain. If it spreads within the risk assessment area it could potentially affect many other similar, threatened, endemic cyprinodontiforms, especially in the Mediterranean. Other impacts are barely studied but the fact that this species if often numerically dominant in both the native and introduced areas suggests that it has overall ecological effects on native species, food webs and ecosystems functioning. Impacts on ecosystem services seem less known but moderate.
Conclusion of the risk assessment	high	medium	The mummichog is a cyprinodontiform fish native to eastern coast of North America, where it is very abundant. It is used in the aquarium hobby and for research and could entry through these and other pathways. It is a very hardy species that tolerates a range of temperatures and salinities, has established in two separate areas of the Iberian Peninsula and it is very likely to establish in most coastal areas of the European Union, if introduced. It is rather a sedentary species that has been shown to spread in the Iberian Peninsula

[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly

[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

			although infrequently and slowly. It seems to already impact endemic, endangered Iberian cyprinodontiforms, with less impacts in ecosystem services and reduced economic costs. If introduced to other Mediterranean areas, it is likely to impact other endemic fauna.

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Austria	-	-	-	-
Belgium	-	-	Yes	-
Bulgaria	-	-	Yes	-
Croatia	-	-	Yes	-
Cyprus	-	-	Yes	-
Czech Republic	-	-	-	-
Denmark	-	-	Yes	-
Estonia	-	-	?	-
Finland	-	-	?	-
France	-	-	Yes	-
Germany	-	-	Yes	-
Greece	-	-	Yes	-
Hungary	-	-	-	-
Ireland	-	-	Yes	-
Italy	-	-	Yes	-
Latvia	-	-	?	-
Lithuania	-	-	?	-
Luxembourg	-	-	-	-
Malta	-	-	Yes	-
Netherlands	-	-	Yes	-
Poland	-	-	Yes	-
Portugal	Yes	Yes	Yes	Yes
Romania	-	-	Yes	-

Slovakia	-	-	-	-
Slovenia	-	-	Yes	-
Spain	Yes	Yes	Yes	Yes
Sweden	-	-	?	-
United Kingdom	-	-	Yes	-

Biogeographical regions of the risk assessment area

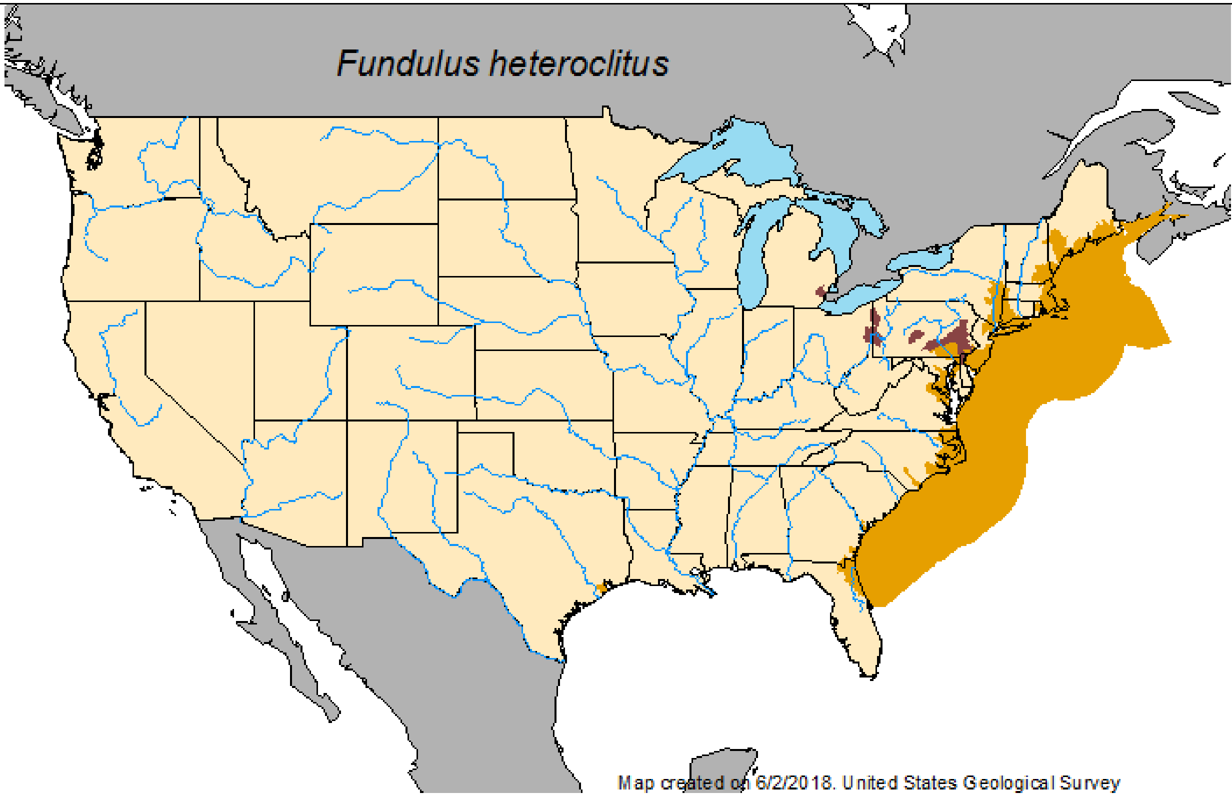
	Recorded	Established (currently)	Established (future)	Invasive (currently)
Alpine	-	-	?	-
Atlantic	-	-	Yes	-
Black Sea	-	-	Yes	-
Boreal	-	-	?	-
Continental	-	-	?	-
Mediterranean	Yes	Yes	Yes	Yes
Pannonian	-	-	-	-
Steppic	-	-	?	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Baltic Sea	-	-	?	-
Black Sea	-	-	Yes	-
North-east Atlantic Ocean	-	-	Yes	-
Bay of Biscay and the Iberian Coast	Yes	Yes	Yes	Yes
Celtic Sea	-	-	Yes	-
Greater North Sea	-	-	Yes	-
Mediterranean Sea	-	-	Yes	-
Adriatic Sea	-	-	Yes	-
Aegean-Levantine Sea	-	-	Yes	-
Ionian Sea and the Central Mediterranean Sea	-	-	Yes	-
Western Mediterranean Sea	Yes	Yes	Yes	Yes

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Actinopterygii, Cyprinodontiformes, Fundulidae *Fundulus heteroclitus* (Linnaeus, 1766) Some frequent synonym names are: *Cobitis heteroclita* Linnaeus, 1766 *Valencia lozanoi* Gómez Caruana, Peiró Gómez & Sánchez Artal, 1984 *Fundulus heteroclitus heteroclitus* (Linnaeus, 1766) *Fundulus heteroclitus macrolepidotus* (Walbaum, 1792) Two subspecies have been traditionally recognized (*Fundulus heteroclitus heteroclitus* and *Fundulus heteroclitus macrolepidotus*) but they have an hybrid zone with clinal variation and are often considered not valid names nowadays (Relyea 1983; Page and Burr 2011; Froese & Pauly 2016; U.S. Fish and Wildlife Service 2017). Common names: mummichog; fúndulo (Spanish); fundulo, peixinho (Portuguese)
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 wild, in confinement or associated with a pathway of introduction]	There are over 40 species of fundulids, all native to North America; Wiley & Ghedotti (2003) and Page & Burr (2011) provide taxonomic information to identify them. Parenti (1981) provides taxonomic keys to identify all cyprinodontiform genera. *Fundulus heteroclitus* is the only fundulid fish naturalised in the European Union, where there are about ten other cyprinondontiform fish present in the wild (see below). However, killifishes (a common term used in general for oviparous cyprinodontiforms) are popular in the aquarium hobby and many other species (including *Fundulus* spp.) are used in Europe (see e.g. https://www.sekweb.org/censo/index.php). Doadrio (2002) and Kottelat & Freyhof (2007) provide extensive information to distinguish *F. heteroclitus* from other similar fish. The only cyprinodontiforms native to the European Union are: *Aphanius baeticus* Doadrio, Carmona & Fernández-Delgado, 2006; *Aphanius fasciatus* (Valenciennes,

	1821); *Aphanius iberus* (Valenciennes, 1846), *Valencia hispanica* (Valenciennes, 1846), *Valencia letourneuxi* (Sauvage, 1880), and *Valencia robertae* Freyhof, Kärst & Geiger, 2014. There are many other cyprinodontiforms endemic from parts of norther Africa, Turkey or the Middle East. The other cyprinodontiforms introduced to the European peninsula are poeciliids, which look considerably different: *Gambusia holbrooki* Girard, 1859, *Gambusia affinis* (Baird & Girard, 1853), *Poecilia reticulata Peters*, 1859, and *Xiphophorus maculatus* (Günther, 1866). All these species live in similar habitats as *Fundulus heteroclitus* and their ecology and life histories are similar. *Fundulus heteroclitus* was misidentified as *Valencia hispanica* and described as a new species (*Valencia lozanoi)* by Gómez, Peiró & Sánchez (1984) in the Iberian Peninsula, before it was realised that is was an introduced species (Fernández-Delgado *et al*., 1986; Morim, 2017).
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	An ecological risk screening of mummichog (*F. heteroclitus*) for the United States was performed by the U.S. Fish & Wildlife Service (U.S. Fish and Wildlife Service, 2017) and concluded that the overall risk assessment was uncertain, due to the lack of a clearly documented history of invasiveness despite the high climate match to much of the contiguous U.S. This risk assessment area does not correspond to the current one (European Union, excluding the outermost regions) but is informative since the climates of the continental U.S. and the European Union are similar and so were our conclusions (high climatic match and establishment risk, potential impact but with low evidence, and short history of invasiveness). In the Iberian Peninsula, where the species has been introduced, there are two published risk assessments (RAs) (Clavero, 2011: Almeida *et al.*, 2013). Clavero (2011) focused mainly on the first stages of invasion (arrival and establishment) developing a specific procedure for the Iberian Peninsula and scored it as 9 in a scale from 0 (minimal risk of invasion) to 25 (high risk), with maximum climatic match (since the species already established). Almeida *et al.* (2013) applied the FISK approach (Fish Invasiveness Scoring Kit), obtaining an outcome of “moderately high” risk for the species. These RAs are highly relevant to the current RA (since they correspond to part of the risk assessment area) and have similar conclusions. In Turkey, where the mummichog has not yet been introduced, a modified version of FISK, the AS-ISK (Aquatic Species Invasiveness Screening Kit), classified the mummichog as of medium risk (Tarkan *et al.*, 2017. This RA is relevant to the current one, given the vicinity of the risk assessment area, and had similar conclusions.
A4. Where is the organism native?	The native range of the species is the Western Atlantic region: from Gulf of St. Lawrence (Canada) to northeast Florida, USA (Froese & Pauly, 2016).

	 Figure A4a. Native (■) and introduced (■) distribution of *Fundulus heteroclitus* in the North American Atlantic coast (Fuller 2018). Note that the introduced area includes some failed introductions (not established).

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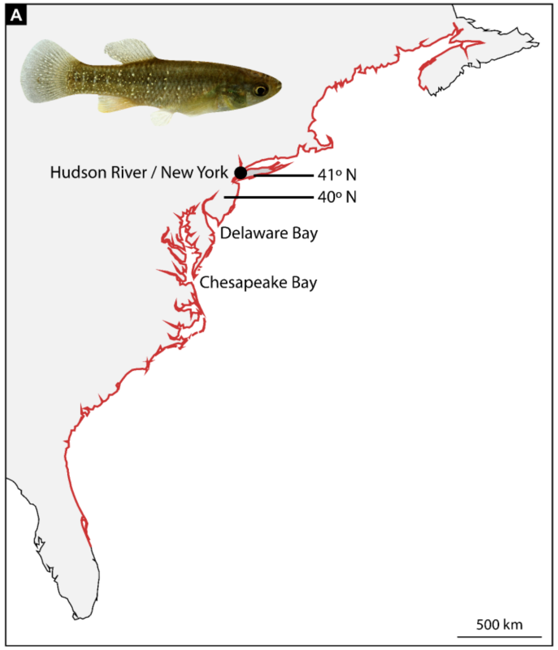

Figure A4b. Native distribution (red line) of Fundulus heteroclitus in the North American Atlantic coast. F. heteroclitus photograph from North American Native Fishes Association (2010). Figure from Morim (2017).
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<td>A5. What is the global non-native distribution of the organism outside the risk assessment area?</td>
<td>There are introductions within the United States such as New Hampshire (Scarola et al., 1987) and western Pennsylvania (Trautman, 1981), possibly as a baitfish; some of these are failed introductions but it is established in the lower Susquehanna and Delaware drainages (U.S. Fish and Wildlife Service, 2017). FAO (2016) and FishBase (Froese & Pauly, 2016) list F. heteroclitus as introduced and established in Hawaii and The Philippines but the NAS database (Fuller, 2018), government webpages, or other sources do not list it as established or recently present in Hawaii (e.g. Englund, 2000, 2002) and The Philippines (e.g. Joshi, 2006; Cagauan, 2007).</td>
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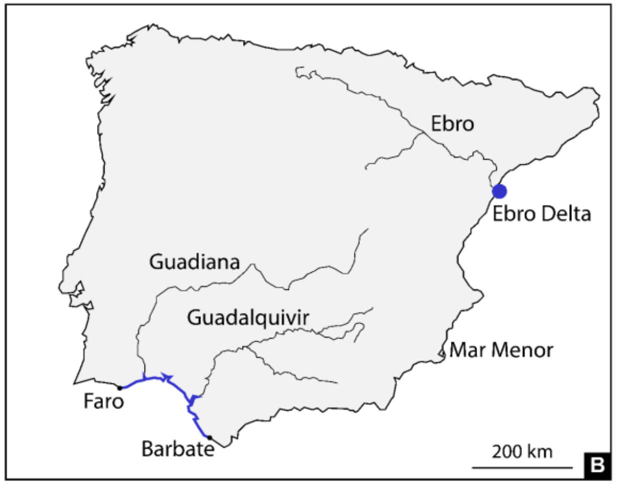
A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established?	 Figure A6. Known alien range (blue line and dot) of *Fundulus heteroclitus* in the Iberian Peninsula. Figure reproduced from Morim (2017). Within Europe, *Fundulus heteroclitus* is only introduced and established in Spain and Portugal (see Fig A6), which falls within the 'Mediterranean' biogeographical region or "North-east Atlantic Ocean" and "Mediterranean Sea" marine regions (EEA, 2012). **Recorded**: List regions Freshwater / terrestrial biogeographic regions: • Mediterranean. Marine regions: • North-east Atlantic Ocean, Mediterranean Sea. Marine subregions: • Bay of Biscay and the Iberian Coast, Western Mediterranean Sea. **Established**: List regions Freshwater / terrestrial biogeographic regions:

	• Mediterranean. Marine regions: • North-east Atlantic Ocean, Mediterranean Sea. Marine subregions: • Bay of Biscay and the Iberian Coast, Western Mediterranean Sea.
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?	**Current climate:** Freshwater / terrestrial biogeographic regions: • Atlantic, Black Sea, Boreal, Continental, Mediterranean, 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. **Future climate:** Freshwater / terrestrial biogeographic regions: • Atlantic, Black Sea, Boreal, Continental, Mediterranean, 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. *Fundulus heteroclitus* originally lives in brackish or salt water and secondarily nearby freshwater, and inhabits sheltered coastal areas such as saltmarshes, tidal creeks, estuaries or bays all year-round (Hardy Jr, 1978; Page & Burr, 2011) along the Atlantic coast of North America between Nova Scotia, Canada and Florida, USA. It withstands a wide range of salinities, from 0 to 120.3 ppm (Griffith, 1974), and temperatures, from -1.5 ºC (Umminger, 1972) to 36.3 ºC (Garside & Chin-Yuen-Kee, 1972), surviving abrupt changes in both parameters (Hardy Jr, 1978; Bulger, 1984). Its native range in eastern North America corresponds to the 'Cfa' and 'Dfb' Köppen-Geiger climate zone (Peel *et al.*, 2007), whereas much of central Europe is in the 'Cfb' zone (similar to 'Cfa'). In the Iberian Peninsula, it has established and spread in the 'Csa' zone. Therefore, it is likely to be able to establish in many European coastal areas in both current and future climates (Fig. A7 and A7b). However, it looks that its spread will be slow, given the lack of many introductions, the slow spread in the Iberian Peninsula, and its sedentary habits (see below). However, it has been recently suggested to be limited by the existence of benthic muddy saltmarsh environments, which are only found near major estuaries or lagoons areas (Morim et al. 2018). The effects of climate change in the progressive warming and salinity of estuaries water might favour its establishment and spread but should not change it much given its wide tolerance and native latitudinal range.

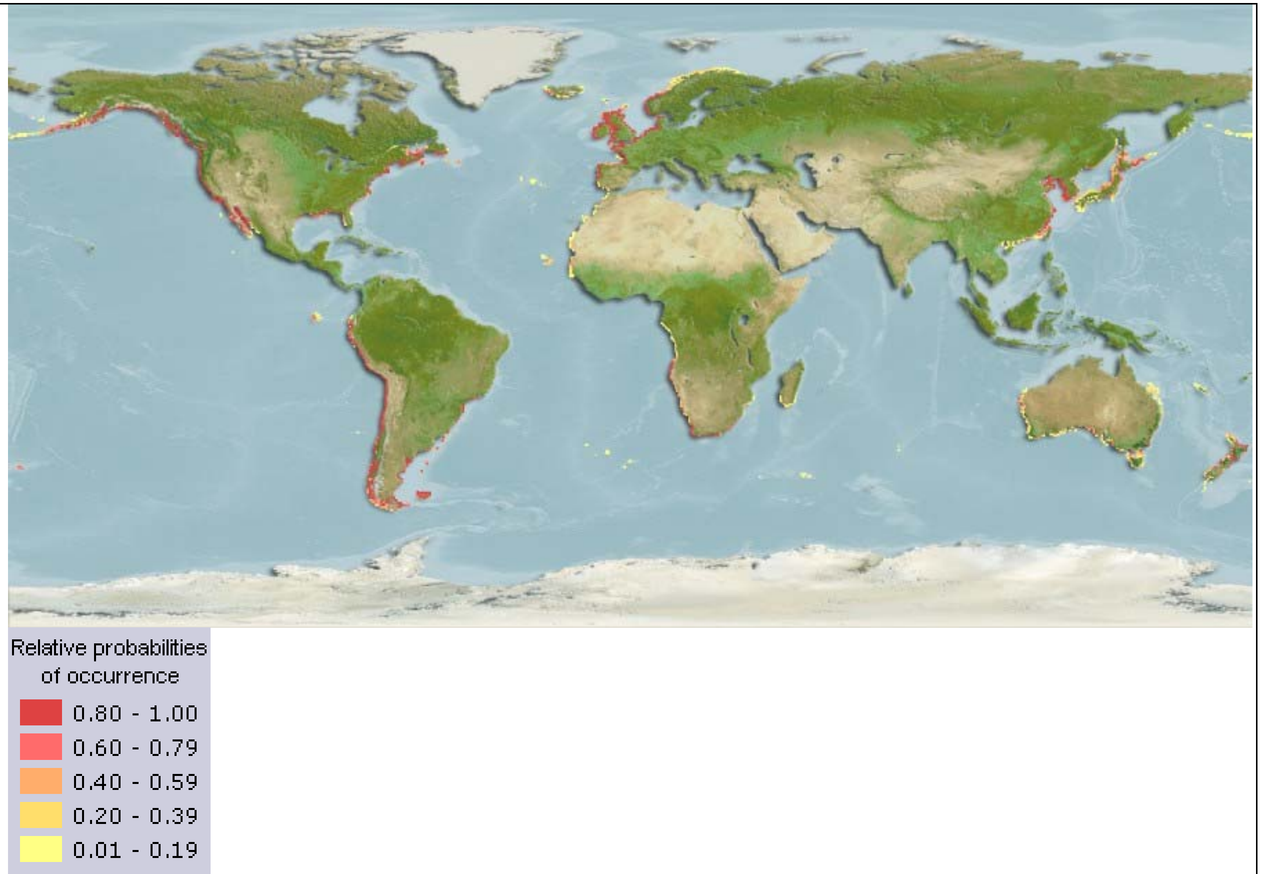
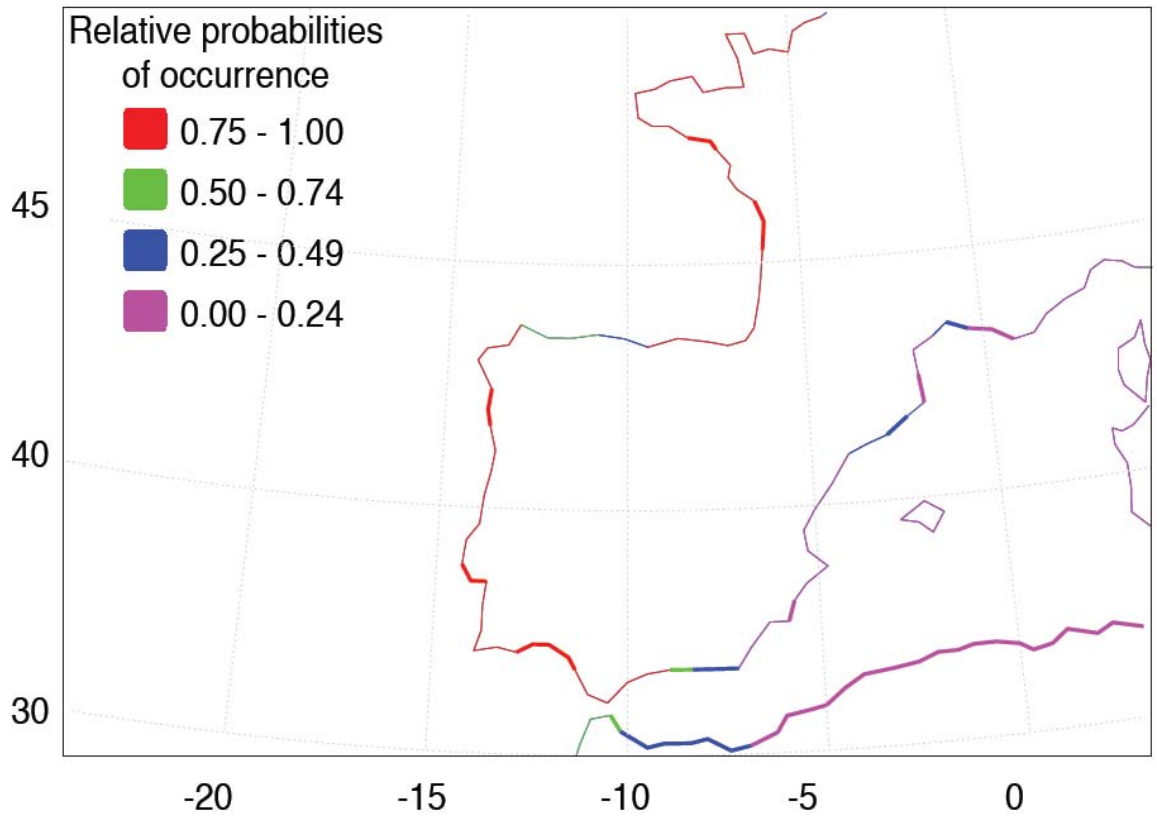


Figure A7. Computer generated potential distribution for *Fundulus heteroclitus* (Mummichog). www.aquamaps.org, version of August 2016. Web. Accessed 4 June 2018.

	 Figure A7b. Probability of occurrence of *Fundulus heteroclitus* in the western European and Mediterranean coastal environments, using AquaMap and environmental predictors, according to Morim et al. (2018; CC BY 4.0 Open Access). Those areas in bold show coastal seabed habitats with a mud content > 10%, where *F. heteroclitus* is very likely to establish, if introduced.
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.	Recorded in the following Member States: Portugal and Spain. Established: Portugal and Spain.

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<td>Morim (2017) states: “The date of introduction in the southern coast Spanish saltmarshes remains uncertain, it was probably introduced between 1970 and 1973 (Fernández-Delgado, 1989). Although Gutiérrez-Estrada et al. (1998) suggested some limitations (see below), they did not exclude the early 1970s as the most likely date of introduction. Almaça (1995) had no suggestion regarding the date of introduction of F. heteroclitus in the Portuguese side of the Guadiana saltmarshes because fish research at the mouth of the Guadiana only took place after 1975, and thus it could have been present for a long time in this region without being reported. By the 1990s, it was already well established in the southwestern coast of Spain, where it could be found almost continuously from the mouth of the Guadiana until the Barbate marshes (Gutiérrez-Estrada et al., 1998). A decade later, its presence was recorded in the Ria Formosa, southern coast of Portugal (at least since 2002 in seabird pellets; e.g., Catry et al., 2006; Paiva et al., 2006) and in the Ebro Delta in the Mediterranean Sea, north-eastern coast of Spain (Gisbert & López, 2007)” (Figure A8).

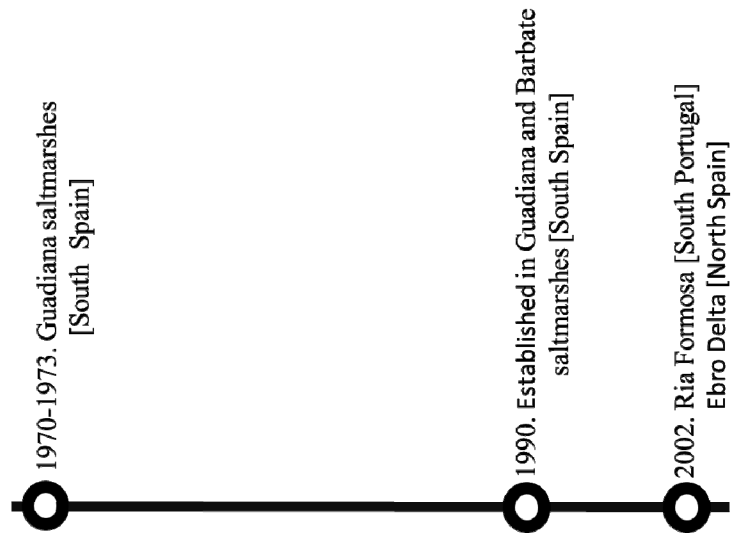

Figure A8. Timeline of observations of Fundulus heteroclitus in Iberian Peninsula.</td>
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<td>A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?</td>
<td>Current climate: This species has a wide latitudinal range in its native distribution (see section A7). It could establish in most EU member states with a marine coast, i.e. Belgium, Bulgaria, Croatia, Cyprus, Denmark, France, Germany, Greece, Italy, Ireland, Malta, the Netherlands, Poland, Romania, Slovenia, the United Kingdom and possibly Estonia, Finland, Latvia, Lithuania, and Sweden.</td>
</tr>
</table>

	Future climate: This species has a wide latitudinal range in its native distribution and climate change should not change much its establishment probability (see section A7). Therefore, under foreseeable climate change it could establish in most EU member states with a marine coast, i.e. Belgium, Bulgaria, Croatia, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Ireland, Latvia, Lithuania, Malta, the Netherlands, Poland, Romania, Slovenia, Sweden and the United Kingdom.
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 existing ecological risks assessments report impacts in Iberian fresh waters but not for the US introductions. This species has barely been introduced outside Europe so there are no impacts reported elsewhere.
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	Freshwater / terrestrial biogeographic regions: • Mediterranean Marine regions: • North-east Atlantic Ocean, Mediterranean Sea Marine subregions: Bay of Biscay and the Iberian Coast, Western Mediterranean Sea. See section A7.
A12. In which EU member states has the species shown signs of invasiveness?	Portugal and Spain.
A13. Describe any known socio-economic benefits of the organism.	*Fundulus heteroclitus* is used as ornamental, as bait in sport fisheries, for biological control agents of mosquito larvae (FAO, 2016) and for scientific research. The species is able to tolerate extreme chemical (contamination) and physical conditions (temperature, salinity, oxygen, etc.) (Hardy Jr, 1978; Bulger, 1984) and is easy to reproduce in captivity. For this reason, mummichog is commonly used in scientific research of stress biology, thermal physiology, toxicology, developmental biology, endocrinology, cancer biology genetics or chronobiology and is considered a model species; it is supposed to be the only freshwater fish species used in a space experiment (Bailey *et al.*, 1996; Hawkins *et al.*, 2003; Law, 2001; Walter & Kazianis, 2001; Winn, 2001; Kent *et al.*, 2009). Gutiérrez-Estrada *et al.* (1998) state that "*F. heteroclitus* is consumed in large quantities by very important commercial fish species, such as large *Sparus aurata* and *Dicentrarchus labrax* (Arias, pers. comm.)." of the Atlantic coast of Spain.

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.”
- The classification of pathways developed by the Convention of Biological Diversity shall 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].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	few	medium	In the Iberian Peninsula (IP), where the mummichog is locally dominant in abundance, the introduction pathways are unclear (Gutiérrez-Estrada *et al.*, 1998; Morim et al. 2018; see below for further details) but might be multiple and transferable to the risk assessment area.

[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

1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	**A) ESCAPE FROM CONFINEMENT** (Pet / aquarium / terrarium) **B) ESCAPE FROM CONFINEMENT** (Research & ex-situ breeding) **C) TRANSPORT – CONTAMINANT** (Contaminated bait) **D) TRANSPORT - STOWAWAY** (Ship/Boat ballast water)		Killifishes (a common term vaguely used mostly for oviparous cyprinodontiforms) are very popular aquarium fish (Wildekamp, 1993), with several existing hobbyist associations (e.g. http://www.bka.org.uk, https://www.sekweb.org/index_en.php). See below for further details. Similarly, *Fundulus heteroclitus* is a model species used extensive in experimental research, including European laboratories. See 1.3b for examples and justification of the current relevance of this pathway. In the USA, the introductions were mostly as bait bucket releases (U.S. Fish and Wildlife Service, 2017) and in Hawaii for mosquito control (FAO, 2016; Froese & Pauly, 2016). The importation of this particular species for mosquito control or bait seems unlikely, but it could be imported as a contaminant in live bait (see below). Its use as bait exists in the risk assessment area as reported in some Spanish websites (e.g. http://www.surfcastingcadiz.com/seccion_cebos/el_fundulo.html) but corresponds to spread (movement of an organism within the risk assessment area) rather than introduction to the risk assessment area, given the definitions above. It has been hypothesized that mummichog was introduced through ballast water in the southern Iberian Peninsula (see below). Finally, this species might be introduced as a stowaway in tanks and containers of live fish importations.
Pathway name:	**A) ESCAPE FROM CONFINEMENT** (Pet / aquarium / terrarium)		
1.3a. Is introduction along this pathway intentional (e.g.	intentional	high	Killifishes (a common term vaguely used mostly for

the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)			oviparous cyprinodontiforms) are very popular aquarium fish (Wildekamp, 1993), with several existing hobbyist associations (e.g. http://www.bka.org.uk, https://www.sekweb.org/index_en.php). Although the mummichog *F. heteroclitus*, which is also called the common killifish, is not a popular species because it is not as beautifully coloured as other species in the group, it is possible that there is importation of this species for the aquarium trade or hobby. Although Maceda-Veiga *et al.* (2013) did not detect this species in some European wholesalers and retailers and its transport and commerce is now forbidden in Spain since it is included in the National black list (Catálogo Nacional de Especies Invasoras), FishBase (Froese & Pauly, 2016) lists *F. heteroclitus* as in the aquarium trade. Moreover. *F. heteroclitus i*s an intertidal spawner and its eggs resist desiccation for several days (Taylor 1999). Therefore, importation from outside Europe for aquarium purposes should be possible and this pathway is intentional (the organism would be imported for trade or use) (see also Fig 1 in the Guidance document).
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	moderately likely	medium	Maceda-Veiga *et al.* (2013) did not detect this species in some European wholesalers and retailers (see 1.3a). However, the mummichog "is the most abundant resident fish in most of the salt marshes on the east coast of the United States" (Teo & Able, 2003). Moreover, it is a small-sized, hardy fish that can be transported in small volumes of water. Therefore, the movement of large numbers seems moderately likely.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	The fish could escape from aquarium fish farms or be released as un undesirable pet (e.g. after growing to a certain size). Aquarium fish are sometimes released in the wild by aquarium hobbyists (e.g. this is probably how the guppy established in thermal springs in Spain

			Hungary and elsewhere) or escape from aquarium facilities. Morim et al. (2018) discuss several possible mechanisms of the first introduction to Europe (southern Iberia) and suggest that aquarium trade is the most likely.
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	moderately likely	medium	The risk of introduction and entry exists.
Pathway name:	**B) ESCAPE FROM CONFINEMENT** (Research & ex-situ breeding)		
1.3b. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional	high	*Fundulus heteroclitus* is an experimental model species used extensively in research. Although “countless mummichogs have been hatched in the laboratory, the species has rarely been bred in captivity, that is, propagated from generation to generation.” and “it is not widely available like the goldfish, is not easily bred in aquaria like the live bearing guppy” (Atz, 1986). Therefore, the specimens used in the laboratory probably originate largely from wild populations or are imported or bought, so the introduction (“movement of the species into the risk assessment area”) is intentional although the entry (“release/escape/arrival in the environment, i.e. occurrence in the wild”) would likely be unintentional. In the Ebro delta, this species might have been introduced “from southwestern Spain for research purposes, since this species was used as a biological model in an Aquaculture Research Centre from 2001 up to middle 2004. Although the wild specimens were found within c. 2 km of the IRTA, containment measures had been undertaken at these research facilities in order to minimize any risk of escape of any developmental stage of *F. heteroclitus* (from egg to adult)” (Gisbert & López, 2007). Other authors are more convinced that the mummichog escaped from this

			research center (Sierra, 2006; Q. Pou-Rovira, personal communication). Examples of recent research using this species in Europe are Tingaud-Sequeira *et al.* (2009), Lombardo *et al.* (2011, 2012), which seem to have obtained the individuals from southern Spain. Its transport and commerce is now forbidden in Spain since it is included in the National black list (Catálogo Nacional de Especies Invasoras), unless a specific permit is given. Therefore, importation from outside Europe either for research or aquarium purposes should not be difficult at present and possible.
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	moderately likely	low	The movement of large numbers is moderately likely.
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	The entry to the Ebro delta was possibly through escapements from an Experimental Research Centre, so it seems moderately likely
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	moderately likely	medium	The risk of introduction and entry seems to clearly exist.
Pathway name:	**C) TRANSPORT - CONTAMINANT** (Contaminated bait)		
1.3c. 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)?	unintentional	high	It could be transported as a contaminant of live bait and this pathway is unintentional.
1.4c. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the	moderately likely	low	*F. heteroclitus* is a small-sized, hardy fish, very abundant in eastern North America. Since live bait (fish and other animals) are transported at the global scale, this species could easily travel as a contaminant.

organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.			
1.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	high	The species is considered to be well adapted to environmental changes as long as a wide range of salinities (0 to 120.3 ppm) and temperatures (-1.5 to 36.3 ºC) (Griffith, 1974; Umminger, 1972; Garside & Chin-Yuen-Kee, 1972). The organism survives abrupt changes in both parameters (Bulger, 1984; Hardy Jr, 1978). It seems possible but unlikely that the species could reproduce during transport.
1.6c. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	medium	It could get unnoticed or unchecked by border controls.
1.7c. How likely is the organism to enter the risk assessment area undetected?	likely	medium	*F. heteroclitus* is a small fish that could easily enter the risk assessment area undetected.
1.8c. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	high	Mummichog is a hardy species so it could survive and establish any time of the year in suitable climates.
1.9c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	unlikely	low	If the bait is for an open aquaculture facility it could escape and reach a suitable habitat
1.10c. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	unlikely	low	The introduction through this pathway seems moderately likely but the entry unlikely
Pathway name:	**D) TRANSPORT - STOWAWAY** (Ship/Boat ballast water)		
1.3d. 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)?	unintentional	high	It could be transported through ballast water (see below) and this introduction is unintentional
1.4d. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also	moderately likely	low	In the southern IP, the mummichog was originally introduced in the marshes of the province of Huelva in the early 1970s, with individuals coming from the northern area (Nova Scotia) of its natural distribution range (Bernardi *et al.*, 1995). The way in which this introduction was accomplished is unclear (Gutiérrez-

comment on the volume of movement along this pathway.			Estrada *et al.*, 1998) but it has been hypothesized that it could have been introduced through ballast water (Sierra, 2006; García-Revillo & Fernández-Delgado 2009, Gonçalves *et al.* 2017), as several invertebrates present in the Guadalquivir river (e.g. *Eriocheir sinensis*, *Rithropanopeus harrisii*, *Haliplanella lineata*) (García-Revillo & Fernández-Delgado 2009). However, there is no direct evidence for this and although introduction of fish with ballast water is frequent (Hutchings, 1992; Williams *et al.*, 1988; Wonham *et al.*, 2000), we found no information of clear introductions or detections in ballast water for mummichog. For example, in their extensive global review, Wonham *et al.* (2000), reported 31 fish species detected in ballast water (but not mummichog) and 24 established introductions attributed to ballast water, which included three cyprinodontid fish species, but not the mummichog. *F. heteroclitus* “is the most abundant resident fish in most of the salt marshes on the east coast of the United States” (Teo & Able, 2003) and thus accidental transport with ballast water in large numbers seems moderately likely, although we found limited evidence of it.
1.5d. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	high	The species is considered to be well adapted to environmental changes such as a wide range of salinities (0 to 120.3 ppm) and temperatures (-1.5 to 36.3 ºC) (Griffith, 1974; Umminger, 1972; Garside & Chin-Yuen-Kee, 1972). The organism survives abrupt changes in both parameters as well (Bulger, 1984; Hardy Jr, 1978). “The single attribute of the mummichog that has been most responsible for its remarkable popularity as a laboratory animal is its hardiness in captivity.” (Atz, 1986).

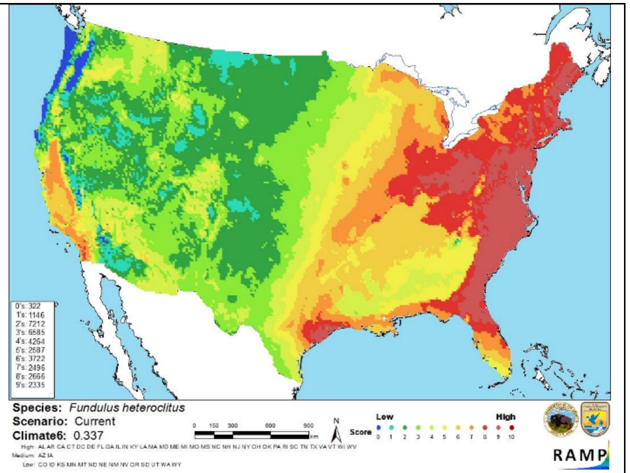
1.6d. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	medium	F. heteroclitus is a small-sized, euryhaline fish so it could survive management practices related to exchanges of ballast water with different salinities.
1.7d. How likely is the organism to enter the risk assessment area undetected?	likely	medium	*F. heteroclitus* is a small-sized fish that can could thus easily enter the risk assessment area undetected.
1.8d. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	high	Mummichog is a hardy species so it could survive and establish any time of the year in suitable climates
1.9d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	If the discharge of ballast water occurs in a suitable habitat for the species (e.g. estuaries or coastal areas), it seems likely to establish. However, this seems to have occurred in few areas so we scored it as moderately likely
1.10d. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	moderately likely	low	Despite the mummichog being the "ideal" fish species to be introduced with ballast water (small, hardy, abundant in a large native area), this has not occurred many times given the few existing introduced populations.
End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	likely	medium	This species is very abundant in the native areas, very hardy, and could be transported by several pathways. Although not widely introduced worldwide, it is likely to entry into the risk assessment area based on all active pathways. The likelihood is similar in different biogeographical regions except the ones without coastal areas (e.g. Pannonian region).
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	likely	medium	Climate change is not expected to affect much this species (see A7) or its overall likelihood of entry into the risk assessment area.

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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very likely	high	*F. heteroclitus* is a very tolerant species in terms of temperature and salinity (Griffith, 1974; Umminger, 1972; Garside & Chin-Yuen-Kee, 1972). Its original range includes much of the east coast of USA and Canada, mainly in brackish or saltwater, and it inhabits sheltered coastal areas such as saltmarshes, tidal creeks, estuaries, or bays. In these coastal habitats, it could easily establish in a wide latitudinal range (see Fig. A7 for a map with the potential distribution). Another climate matching map of the species in the USA is available (Fig. 1.13), although it does not seem very reliable since mummichog is mostly a brackishwater species.

			 Figure 1.13. Map of climate matches for *Fundulus heteroclitus* in the contiguous United States based on source locations reported by Fuller (2018) and GBIF. Figure obtained from U.S. Fish and Wildlife Service (2017).
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	very likely	high	*F. heteroclitus* is very tolerant to diverse abiotic conditions (See comments to Q1.13 above and elsewhere) and it has already established in the risk assessment area (Portugal and two separate areas in Spain), and it could likely establish in many other countries.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	widespread	high	*F. heteroclitus* prefers salt marshes with brackish water but can tolerate freshwater and a range of temperatures so it could establish along much of the European coast and most climates of the risk assessment area. It seems to be limited by the existence of benthic muddy saltmarsh environments, which are only found near major estuaries or lagoons areas (Morim et al. 2018) (see A7 above).

1.16. 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?	NA	high	There is no known particular species necessary for critical stages in its life cycle.
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very likely	high	*F. heteroclitus* "is the most abundant resident fish in most of the salt marshes on the east coast of the United States" (Teo & Able, 2003) and has established and is abundant in some parts of the Iberian Peninsula (Gutiérrez-Estrada *et al.*, 1998) so competition is unlikely to prevent establishment.
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very likely	high	*F. heteroclitus* "is the most abundant resident fish in most of the salt marshes on the east coast of the United States" (Teo & Able, 2003) and has established and is abundant in some parts of the Iberian Peninsula (Gutiérrez-Estrada *et al.*, 1998) so biotic interactions are unlikely to prevent establishment. There are generic studies on infectivity of *A. invadans* (epizootic ulcerative syndrome) and viral haemorrhagic septicaemia virus (ectoparasites) (Johnson *et al*., 2004; Gagné *et al.*, 2007; Bailly, 2009). No studies have been found of parasites on the Mummichog in the risk assessment area.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very likely	medium	Control experiences of the species by means of passive methods such as fishing net or pots have not served to limit the establishment of the species in the eastern Iberian Peninsula (Pou i Rovira, 2008). If released intentionally or accidentally, it is likely to establish.
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	moderately likely	low	In Spain, the transport and commerce of this species is forbidden since it is included in the National black list (Catálogo Nacional de Especies Invasoras). However, current management

			practices in Spain have not limited the establishment of new fish species in the last 20 years, since there is much illegal or unnoticed fish movement. This is probably the case in other European countries.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	likely	medium	This is a very abundant, small-sized, hardy fish, with ideal properties to resist eradication campaigns in the risk assessment area.
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	very likely	high	This is a very abundant, small-sized, hardy fish, with ideal properties to facilitate its establishment in the risk assessment area. *F. heteroclitus* are gregarious and live up to 4 years. It reaches sexual maturity about 35 mm SL and about 1 year. Spawns in April-June in European waters. Eggs are spawned one by one, adhere to vegetation by filaments, and hatch in 12-14 days (Kottelat & Freyhof, 2007). *F. heteroclitus* feed mostly on small crustaceans and polychaetes. Fish longer than 30 mm also ingest considerable living plants (Kneib & Stiven, 1978). Kneib & Parker (1991) conducted experiments about gross food in larval mummichogs and they suggested that natural prey concentration is decisive for fish growth. It feeds at surface, mid-water, and off bottom, mainly at high tide during daylight, but also opportunistically (Abraham, 1985). In its native area, *F. heteroclitus* needs an annual reproductive cycle containing lunar and semilunar spawning cycles in January (Hsiao *et al.*, 1994). The species shows a large primary spawning peak

			in spring followed by a smaller secondary one in mid-summer (Kneib & Stiven, 1978). The eggs are usually located in places covered by high spring tides, usually in sand (Taylor, 1986). Eggs are normally incubated in the air (essential for survival) until the next spring tide. Decreases in salinity from spring rains can decrease the success of fertilization and increase larval mortality (Able & Palmer, 1988). *F. heteroclitus* in aquaria may lay up 40 egg/day depending on size, with some females spawning almost daily throughout the season (Foster, 1967). In field populations, conditions are rarely optimal so that the number of eggs spawned per day is reduced (Kneib & Stiven, 1978). Hatching of most eggs was estimated to occur in May. The main growing season is from April to September. The species grows rapidly with females sexually mature (30-35 mm) in 5-6 months. Mortality in females increases dramatically after the first reproduction at the end of the second growing season (Kneib & Stiven, 1978).
1.23. How likely is the adaptability of the organism to facilitate its establishment?	very likely	high	This is a very adaptable species (to brackish waters), what is likely to facilitate its establishment. In its native area (North America), *F. heteroclitus* are non-migratory, and the movement of individuals is usually localised, limited to relatively small areas, with some individuals occasionally dispersing over longer distances. The organism makes small movements between summer and winter habitats with lower salinity areas (Smith & Able, 1994). There are several possible advantages to remaining in the saltmarsh

			pools during the winter. They are shallow, which allows rapid increases in water temperature. On sunny days in winter, *F. heteroclitus* are active, and temperature increases may be high enough to allow feeding during the day. Small increases in water temperature have been shown to increase *F. heteroclitus* metabolism, especially at water temperatures below 5 °C. In addition, there is little water flow in marsh pools in winter, so fish are not forced to expend energy maintaining their position as they would in the tidal creek (Smith & Able, 1994).
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	likely	medium	Studies of genetic diversity of *F. heteroclitus* in Spain were made by Bernardi *et al.* (1995) and Morim et al. (2018). Bernardi *et al.* (1995) have tried to determine from which of the American populations the Spanish individuals are derived. Their results seem to indicate a low genetic diversity for the Spanish population similar to a northern population of North America. Morim et al. (2018), including a sample from the Ebro delta, confirmed the lack of genetic structure and the likely introduction of a few individuals However, the species has established and is abundant in some parts of the Iberian Peninsula.
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	moderately likely	low	The species is already established and abundant in the Iberian Peninsula but has almost not established introduced populations in other places worldwide; this seems more related to its transport probability and propagule pressure rather than its establishment capacities. It is tolerant to a variety of environmental conditions and very abundant in its native area.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur?	unlikely	medium	See comments provided to Q1.25. If introduced, it is likely to establish.

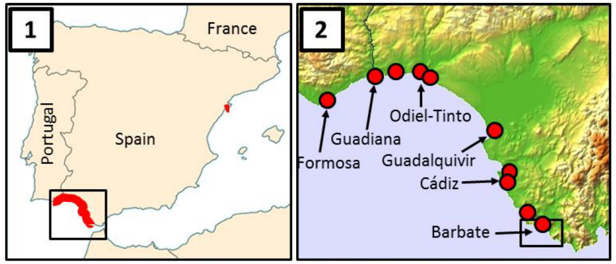
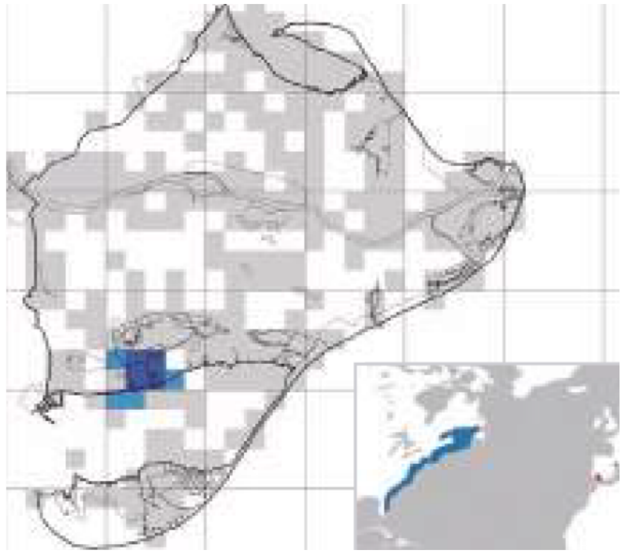
Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.			
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	very likely	high	This is a very abundant, small-sized, hardy fish, with ideal properties to facilitate its establishment in most coastal areas of the risk assessment area. It has not a long history of introductions but it is established and abundant in the Iberian Peninsula. If introduced, it is likely to establish in the following Freshwater / terrestrial biogeographical regions under current climate: Freshwater / terrestrial biogeographic regions: Atlantic, Black Sea, Boreal, Continental, Mediterranean, and Steppic. It is likely to establish in the coastal area of the four marine regions (i.e. Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, and Black Sea). See A7 for further info.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	very likely	high	This is a species with a wide latitudinal range in its native area and tolerant of contrasting temperatures and different abiotic factors so climate change should not affect it much. Therefore, climate change should not affect (possibly reinforce) its likelihood of establishment, which is already high much of the coastal areas of the risk assessment area.

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by **natural** means? (Please list and comment on each of the mechanisms for natural spread.)	minor	high	There has been natural spread within the two introductions in the Iberian Peninsula. In the southern Iberian Peninsula, it has spread slowly since its introduction supposedly in the 1970s (Fig. 2.1a), presumably by natural dispersal, since this species is a euryhaline species that has been shown to be able to use marine environment as dispersal routes (Blanco-Garrido & Clavero, 2016). In a 1-year mark-recapture study in Canada, 97% of recaptured fish were within 200 m of the point of initial release, whereas the rest moved distances ranging from 600 to 3600 m (Skinner *et al.*, 2005). Similarly, since its introduction in a single site of the Ebro delta in 2005 it has spread slowly in the delta (Fig. 2.1b).

			 Figure 2.1a. Distribution and recent expansion of the mummichog (*Fundulus heteroclitus*) in the Iberian Peninsula. 1) Current distribution of the mummichog in the Iberian Peninsula marked with a red line. 2) Main population nuclei of the mummichog in southern Iberian Peninsula (reproduced from Blanco-Garrido & Clavero, 2016).  Figure 2.1b. Distribution of the mummichog (*Fundulus heteroclitus*) in the Ebro delta ca. 2012 (the

			native distribution in North America is also shown) (reproduced from López *et al.*, 2012). Mummichogs make daily tidal migrations between the intertidal marsh surface and adjacent channel and pond habitats (Butner & Brattstrom, 1960; Weisberg & Lotrich, 1982) and, as a result, are hypothesized to play an important role in the export of marsh production to the open estuary (Kneib, 1997). Despite these movements, mummichogs are thought to have a highly restricted summer home range of only 36 m (Lotrich, 1975). However, it was found that in a restored salt marsh, YOY and adults primarily used the shallow subtidal and intertidal areas of the created creek, the intertidal drainage ditches, and the marsh surface of the restored marsh but not the larger, first-order natural creek. At low tide, large numbers were found in the subtidal areas of the created creek; these then moved onto the marsh surface on the flooding tide. Elevation, and thus hydroperiod, appears to influence the microscale use of the marsh surface. So in other studies the home range of adults and large YOY has been estimated to be 15 ha at high tide, much larger than previously quantified (Teo & Able, 2003). There was strong site fidelity to the created creek at low tide. The habitat uses and movement patterns of the mummichog appeared similar to that reported for natural marshes (Teo & Able, 2003).
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	minor	medium	The two intentional pathways of introduction and entry analysed above (ESCAPE FROM CONFINEMENT: Pet / aquarium / terrarium; ESCAPE FROM CONFINEMENT: Research & ex-situ breeding) might also explain “spread” within the

			risk assessment area but should not be considered as such according to the instructions above. For instance, one of these two pathways would explain the introduction to the Ebro Delta (transport by car/road from southern Spain) but it is "intentional anthropogenic "spread" via release or escape [and] should be dealt within the introduction and entry section" (see above). The slow recent spread in the southern Iberian Peninsula was suggested to be most probably by natural spread through the sea. Although a human-assisted expansion is less likely it is also possible, e.g. through bait releases (Blanco-Garrido & Clavero, 2016, Q. Pou_Rovira, personal communication).
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	**TRANSPORT - CONTAMINANT** (Contaminated bait) **TRANSPORT - STOWAWAY** (Ship/Boat ballast water)		See below for justification and some information on the specific origins and end points of the pathways.
Pathway name:	**A) TRANSPORT - CONTAMINANT** (Contaminated bait)		
2.3a. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	both	high	It could be transported as a contaminant of other taxa used for aquaculture or angling. Anglers are frequent nearby sites in Spain where mummichog has been introduced and is abundant and could easily be used as bait and released (Q. Pou-Rovira).
2.4a. 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	moderately likely	medium	A few fish could originate a viable population that would spread along this pathway.

course of one year?			
2.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	high	Very hardy fish (see Q 1.5d and elsewhere). It is likely to survive. Reproduction during passage along the pathway seems unlikely.
2.6a. How likely is the organism to survive existing management practices during spread?	moderately likely	medium	Very hardy fish; moderately likely to survive existing management practices during spread
2.7a. How likely is the organism to spread in the risk assessment area undetected?	moderately likely	medium	*F. heteroclitus* is a small-sized fish that can could thus easily spread the risk assessment area undetected.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	moderately likely	medium	It could spread to any saltmarsh or estuary nearby, which are widespread but a quite specific habitat. Fig. 2.8a shows the main salt marshes in the European Union, where it could spread.

			 Figure 2.8a. Distribution of saltmarsh in Europe (reproduced from Boorman, 2003).
2.9a. Estimate the overall potential for spread within the Union based on this pathway?	slowly	medium	Given the case of the Iberian Peninsula, it is quite likely that the species will spread further into Europe but quite slowly and not necessarily with this pathway
Pathway name:	**B) TRANSPORT - STOWAWAY** (Ship/Boat ballast water)		
2.3b. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	unintentional	high	It could be transported through ballast water within the risk assessment area and this pathway is unintentional
2.4b. 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?	moderately likely	medium	Accidental transport with ballast water within the risk assessment area seems moderately likely. We found no direct evidence of transport or introduction of F. heteroclitus through ballast water (see Q 1.4d). However, the mummichog is abundant in southern Spain, where boats enter the Guadalquivir to mostly

			discharge containers in Seville. Therefore these boats export ballast water and stowaway species rather than import them (García-Revillo & Fernández-Delgado 2009) and could favour spread to other European ports and coastal areas.
2.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	high	Very hardy fish. See Q 1.5b and elsewhere. Reproduction during passage along the pathway seems unlikely.
2.6b. How likely is the organism to survive existing management practices during spread?	moderately likely	medium	*F. heteroclitus* is a small-sized, euryhaline fish so it could survive management practices related to exchanges of ballast water with different salinities or other management practices.
2.7b. How likely is the organism to spread in the risk assessment area undetected?	moderately likely	medium	It should not take very long to detect if there are fish surveys in the region but it could take months to years if not. It would probably spread slowly.
2.8b. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	likely	high	Its arrival location would probably be quite suitable and it could spread to any saltmarsh or estuary nearby.
2.9b. Estimate the overall potential for spread within the Union based on this pathway?	slowly	medium	Given the information available (worldwide history and the case of the Iberian Peninsula), it seems quite likely that the species will spread further into Europe but quite slowly and not frequently.
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	difficult	high	If introduced and established in the risk assessment area, it would likely be difficult and probably impossible to contain *F. heteroclitus* to avoid further spread because this species generally occupies large, open areas (mostly estuaries, coastal lagoons, or similar). When detected as established it would have probably occupied already a considerable area, since

			it is a small fish with rapid maturation (one year after hatching in southern Iberia), relative long reproductive season (although mostly in March and April in southern Iberia), and high densities (Fernández-Delgado 1989).
2.11. Estimate the overall potential for spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	slowly	high	Given the wide latitudinal range of this species in the native area (see Q A.4), it might spread to many of them, but quite slowly and infrequently (as discussed above). As indicated elsewhere, it could spread to most biogeographical regions of the European Union, namely the Atlantic, Black Sea, Boreal, Continental, Mediterranean, and Steppic Freshwater / terrestrial biogeographic regions and the four marine regions (i.e. Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, and Black Sea). See A7 for further info.
2.12. Estimate the overall potential for spread in relevant biogeographical regions in foreseeable climate change conditions	slowly	high	This is a species with a wide latitudinal range in its native area and tolerant of contrasting temperatures and different abiotic factors so climate change should not affect it much. Therefore, climate change should not affect much (possibly reinforce) its potential for spread in the many biogeographical regions where it could spread (see Q2.11).

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is 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?	minor	high	*Fundulus heteroclitus* was introduced to Hawaii and The Philippines but apparently did not establish there, so there is virtually no other introduced populations than those in Spain and Portugal and a few drainages in the USA, where there are no known reported impacts
2.14. 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)?	major	low	The Iberian Peninsula has three endemic, threatened cyprinodontiforms: *Aphanius iberus* (Valenciennes, 1846), *Valencia hispanica* (Valenciennes, 1846), and the recently described *Aphanius baeticus* Doadrio, Carmona y Fernández-Delgado, 2006. *Aphanius baeticus* in southern Spain and *Aphanius iberus* in the Mediterranean Spain occupy a very similar habitat than *F. heteroclitus*. *F. heteroclitus* poses a potential threat by competition and/or predation of the endemic species, and may act synergistically with habitat destruction resulting in a more profound negative impact (Bernardi *et al.*, 1995;

			Doadrio *et al.*, 2002; Elvira, 1996; Elvira & Almodóvar, 2001; Fernández-Delgado, 1989; García-Berthou *et al.*, 2007; García-Llorente *et al.*, 2008; Leunda, 2010; Oliva-Paterna *et al.*, 2006; Planelles & Reyna, 1996; Morim 2017). Mummichog is often numerically dominant in western Andalusian coastal marshes, and it is suspected that it may have negatively affected native endemic species as the endangered Andalusian toothcarp, *Aphanius baeticus* (Gutiérrez-Estrada *et al.*, 1998). According to Gutiérrez-Estrada *et al.* (1998): "If mummichog were outcompeting other species, the mechanisms of this potential exclusion have not been directly evaluated and remain unknown. However, direct predation does not seem to be a factor because *F. heteroclitus* consumes only invertebrates and plants in the study area (Hernando, 1975; Arias & Drake, 1986). In addition, the competition for food does not seem to be a decisive factor due to the enormous productivity of the areas where it is found. Therefore, perhaps, the competition for space could be the best explanation for this apparent segregation observed for mummichog and other fish species in the study area". "It is difficult to evaluate the precise ecological consequences of the mummichog introduction in southern Iberia, especially due to the fact that the original environmental conditions existing in the area where it was introduced are unknown. However, it is probable that some effects may have been negative. Some local fish species may have been displaced". It seems to be affecting *Aphanius iberus* in the Ebro delta and could spread to freshwaters where *V. hispanica* inhabits (López *et al.*, 2012).

			F. heteroclitus is often numerically dominant in both the native area and its introduced area in southern Iberia.
2.15. 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?	major	low	The potential invaded area of the species is limited to coastal saline areas. The impact in the introduced areas has not been realised since it has spread recently to new areas. It is likely to decrease the conservation status of some these threatened species by decreasing their abundance and range and possibly their genetic diversity. If mummichog arrives to new areas of the risk assessment area, it could affect other threatened species, such as *Aphanius fasciatus* in Mediterranean coastal areas, *Valencia* spp. in Greece and others. The mummichog, *Fundulus heteroclitus*, "is the most abundant resident fish in most of the salt marshes on the east coast of the United States, and, as a result, is a key ecological component" (Teo & Able, 2003). Since it is very abundant in some Iberian populations, it is likely to also play a key ecological role in the food web and ecosystem functioning and change current structure.
2.16. 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?	major	medium	*F. heteroclitus* mostly inhabits protected areas (e.g. the Doñana National Park or the Ebro Natural Park in Spain, where *F. heteroclitus* is now abundant). It seems to be clearly affecting two threatened species: - *Aphanius baeticus* (EN), - *Aphanius iberus* (EN), The zones inhabited by *F. heteroclitus* are mostly transitional areas according to the Water Framework Directive (WFD); the effects of mummichog for the

			WFD assessment are largely unknown.
2.17. 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?	major	medium	The introduction and spread of *Fundulus heteroclitus* in the saltmarshes of the risk assessment area might affect a multitude of native, threatened species and saltmarsh habitat types. In addition, all the marshes of the European Union usually have a degree of protection due to their high ecological uniqueness. The species potentially impacted are included in the red list or are endemic species (Kottelat & Freyhof, 2007; Freyhof & Brooks, 2011), namely: - *Aphanius almiriensis* (CR), - *Aphanius baeticus* (EN), - *Aphanius fasciatus* (LC), - *Aphanius iberus* (EN), - *Valencia hispanica* (CR), - *Valencia letourneuxi* (CR) - *Valencia robertae* (not yet evaluated) (CR = Critically Endangered, EN = Endangered, LC = Last Concern and VU = Vulnerable) If it penetrates to low salinity stenohaline environments, it could also affect *Gasterosteus aculeatus* (LC) or *Cobitis paludica* (VU), among many others. Saltmarsh habitat types are protected under Directive 92/43/EEC on the conservation of natural habitats and wild flora and fauna and specific national or regional legislation.
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	minor	high	*Fundulus heteroclitus* was introduced to Hawaii and The Philippines but apparently did not establish there, so there is virtually no other introduced populations

			than those in Spain and Portugal and a few drainages in the USA, where there are no known reported impacts
2.19. 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)?	moderate	low	The impact of mummichog on ecosystem services is caused by possible changes to the food web due resource competition, predations, or spread of disease. This can possibly lead to diminishing of the provisioning of native species for fisheries and quality of nursery habitats. It can also cause changes in ecosystems structure and species composition that make it attractive for recreation, wild life watching etc. Provisioning: In southern Spain, there have been probably negative impacts in traditional prawn fishery yields, which are known to be heavily consumed by mummichog (Arias & Drake, 1986; U.S. Fish and Wildlife Service, 2017). We found no published information on this question but some impacts on regulation and maintenance (e.g. given species abundance and important ecological role) and cultural services are likely.
2.20. 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?	moderate	low	If it spreads to other European areas, the impacts should be similar than in the Iberian Peninsula but affecting many other species, ecosystems and human populations.
Economic impacts			
2.21. 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	minor	high	*Fundulus heteroclitus* was introduced to Hawaii and The Philippines but apparently did not establish there, so there is virtually no other introduced populations than those in Spain and Portugal and a few drainages in the USA, where there are no known reported impacts

2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	minor	low	The economic costs of the mummichog in the Iberian Peninsula has not yet been evaluated but see Q 2.19
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	moderate	low	They have not been well evaluated but do not seem very large. It could affect coastal areas where there are fisheries or aquaculture by changing ecosystem structure and functioning.
2.24. 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)?	moderate	low	The economic costs associated with the management of the mummichog in the Iberian Peninsula have not yet been evaluated. However, money is spent in monitoring and control the invasive species and to implement further conservation plans for native and endemic, threatened species (maintaining captive stocks, restocking, etc.).
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	moderate	low	While no cost estimates for mummichog are available, information on other species can be used as a proxy. Britton *et al.* (2008) list the cost of eradication by different means and site of *Pseudorasbora parva* and estimated cost of 1.9-7.9 £/m^2 in UK ponds. Given the large, open areas occupied by *F. heteroclitus* in Spain, eradication is probably not feasible in most sites but would cost hundreds of thousands of euros.
Social and human health impacts			
2.26. 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).	minimal	medium	Harmless to humans according to FishBase (Froese & Pauly, 2016). However, possible wider societal impacts could arise if the invasion has negative impacts on fisheries and other ecosystem services (see 2.19) and starts to threaten local livelihoods.
2.27. How important is social, human health or other impact (not directly included in any earlier categories)	minimal	medium	No information has been found on this issue.

caused by the organism in the future for the risk assessment area.			
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	moderate	low	From Johnson *et al.* (2004): "We explored the infectivity of *A. invadans* (WIC strain) when inoculated into four commonly occurring species: Atlantic menhaden, striped killifish, *Fundulus majalis* (Walbaum), mummichog *F. heteroclitus* (L.), and *hogchoker*, *Trinectes maculatus* (Bloch & Schneider). […] Mummichogs experienced a lower prevalence of lesions compared with the other species. […]" Infection with *A. invadans* (epizootic ulcerative syndrome) is an OIE-reportable disease. From Gagné *et al.* (2007): "Viral haemorrhagic septicaemia virus (VHSV) was isolated from mortalities occurring in populations of mummichog, *Fundulus heteroclitus*, stickleback, *Gasterosteus aculeatus*, brown trout, *Salmo trutta*, and striped bass, *Morone saxatilis*, in New Brunswick and Nova Scotia, Canada." Viral haemorrhagic septicaemia virus is an OIE-reportable disease. From Bailly (2009): "*Caligus rufimaculatus* Wilson C.B., 1905 [via synonym] (parasitic: ectoparasitic) *Ergasilus funduli* Krøyer, 1863 [via synonym] (parasitic: ectoparasitic) *Ergasilus manicatus* Wilson C.B., 1911 [via synonym] (parasitic: ectoparasitic) *Homalometron pallidum* Stafford, 1904 [via synonym]

			(parasitic: endoparasitic) *Lernaea cyprinacea* Linnaeus, 1758 [via synonym] (parasitic: ectoparasitic) *Lernaeenicus radiatus* Le Sueur, 1824 [via synonym] (parasitic: ectoparasitic) 6 *Swingleus ancistrus* Billeter, Klink & Maugel, 2000 [via synonym] (parasitic: ectoparasitic)”
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	NA		
2.30. 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?	moderate	low	Mummichog is abundant in some parts of southern Spain so predators or other enemies do not control their populations.

ANNEXES

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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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[9]	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 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.

[9] Not to be confused with „no impact".

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.*. 2017)

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[10]	**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.*

[10] 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.

	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** 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*

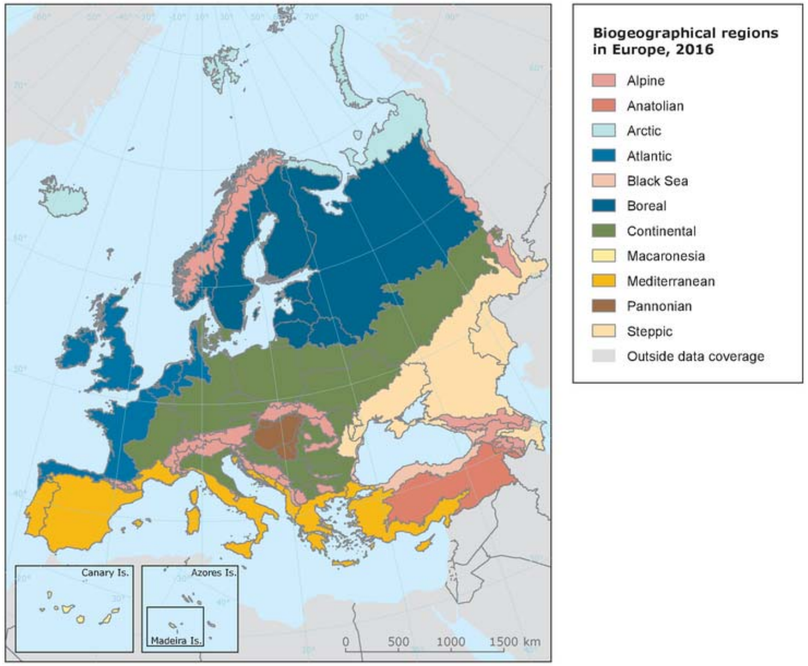
			composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living 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.*

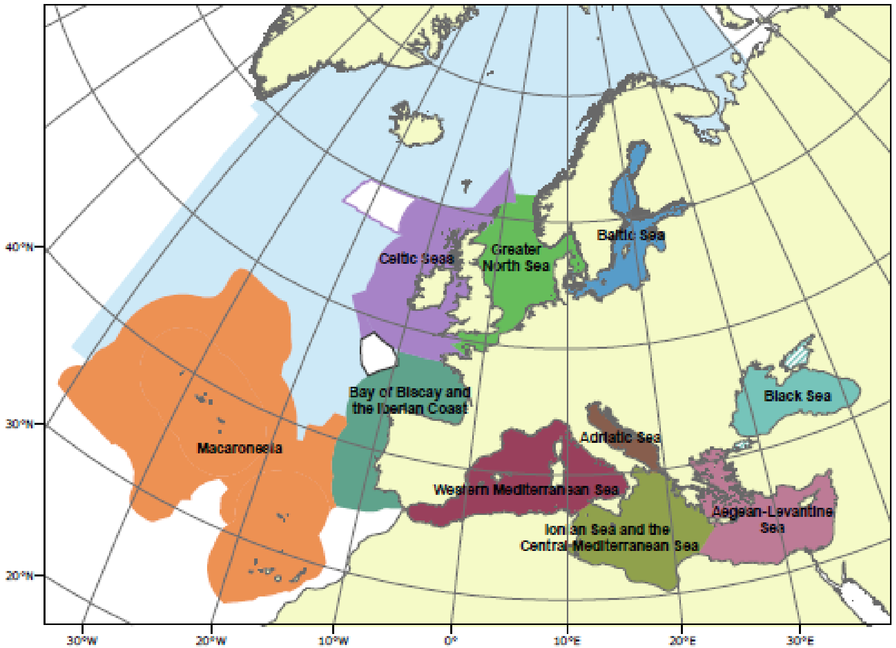
ANNEX V EU Biogeographic Regions and MSFD Subregions

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 with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	Mummichog
Species (common name)	*Fundulus heteroclitus*
Author(s)	Emili García-Berthou & Juan Diego Alcaraz-Hernández
Date Completed	23/10/2018
Reviewer	Peter Robertson

Summary

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.

Prevention

Fundulus heteroclitus has been introduced and has established populations in two separate areas of the Iberian Peninsula. The introduction pathways are unclear but might be multiple, likely escape from confinement (e.g. aquarium, research and ex situ breeding), transport – contaminant (contaminated bait), and transport - stowaway (ship/boat ballast water). Therefore, the main measure to achieve prevention is improving awareness and managing these potential multiple pathways.

Early detection

This species is easy to distinguish from other fishes but rather with a "fast" life-history strategy (i.e. small-sized, early maturation, and ability for populations to rapidly increase), very tolerant to diverse abiotic conditions (salinity, temperature, oxygen concentration) and lives in large, open habitats (estuaries, coastal lagoons, and saltmarshes). Therefore, it could easily form large populations before being detected and eradication would be impossible with current technologies, unless it is a very small, closed habitat (which is not typical of the mummichog). Early detection is thus essential to avoid establishment and reproduction in the open habitats typical of this species and monitoring of suitable habitats in areas close to existing populations seems important. Environmental DNA (eDNA) and citizen science might help early detection.

Eradication

Eradication (e.g. with rotenone) will only be feasible if it is a very recent population (i.e. early detection) in a small or enclosed habitat (e.g. channel or pond). When established in its typical habitat (coastal lagoons and estuaries) eradication will not be feasible with current technologies.

Management

Prevention, early detection, and eradication are the most effective tools for preventing further spread. Mechanical removal might help to control populations in sensible habitats (e.g. where endangered species coexist) but is unlikely to be effective in the long term. Biological control by predatory fish has been suggested as a control method for similar situations but is likely to have wider environmental impacts.

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	**Improving awareness and best practice guidance for key stakeholder groups** *Fundulus heteroclitus* has been introduced and has established populations in two separate areas of the Iberian Peninsula. The introduction pathways are unclear but might me multiple, likely escape from confinement (e.g. aquarium, research and ex situ breeding), transport – contaminant (contaminated bait), and transport - stowaway (ship/boat ballast water). Therefore, the main measure to achieve prevention is improving awareness and managing these potential multiple pathways.	Improving awareness and best practice guidance for key stakeholder groups such as aquarium hobbyists, anglers, and researchers might help to prevent further introductions. Zogaris (2017a, 2017b) lists the following costs for implementing such a program of measures in Kentucky, USA (from Mahala, 2008): a) Development of an alien invasive education specifically for the state: $15,000/year; b) Target and educate key groups: $23,000/year; c) Identify and secure outside funding to develop, maintain and continue the education/awareness program: $250/year; d) Assess public and stakeholder awareness with surveys: $5,000/year; e) Provide programs to assist against entry of species by appointing a coordinator position $5,800/year; f) Annual review and update of plan to address gaps and needs (study, review): $1,000/year. g) In addition, the plans call for the funding of scientific meetings, dissemination and building alliances among stakeholders (estimated costs of meetings etc: $6,000/year). These education programs should be evaluated rather than assuming they are successful (see e.g. Cameron et al. 2013).	Medium

Methods to achieve prevention	**Managing pathways**. Including reducing the likelihood of escapement from confinement (aquarium, research and ex-situ breeding) and introductions as contaminant or stowaway.	The costs of managing the pet/aquarium trade and reducing deliberate introductions" might be similar than for *Gambusia* spp., which were "roughly estimated to be medium for the EU (< €50k?)" by Verreycken & Copp in 2017 (Roy et al. 2018). Extensive information on ballast water management is available elsewhere (e.g. http://www.imo.org). Lucy and Tricarico (in Roy et al. 2018) estimate the cost of Ballast water management systems as of "up to US $5 million/ship with running costs of up to 2-3% of total operational costs for maintenance and management of chemical, filtration units or UV ballast water treatment systems (http://www.ballastwatermanagement.co.uk)."	Medium
Methods to achieve eradication	**Early detection and monitoring**	This species is easy to distinguish from other fishes but rather with a "fast" life-history strategy (i.e. small-sized, early maturation, and ability for populations to rapidly increase), very tolerant to diverse abiotic conditions (salinity, temperature, oxygen concentration) and lives in large, open habitats (estuaries, coastal lagoons, and saltmarshes). Therefore, it could easily form large populations before being detected and eradication would be impossible with current technologies, unless it is in a very small, closed habitat (which is not typical of the mummichog). Early detection is essential to avoid establishment and reproduction in the open habitats typical of this species. Therefore, monitoring is	Medium

		important to detect these and other invasive species. Costs of monitoring small freshwater fish depend on a number of factors (spatial and temporal extent, method, country, etc.) but should be rather ow to medium (< €5k to €50k)" (Roy et al. 2018). Environmental DNA (eDNA) might also be very helpful for early detection (Thomsen et al. 2012, Takahara et al. 2013, Davison et al. 2017). According to Zogaris (2017a), "costs of initial set up (laboratory work, staff, and equipment) for a single laboratory of a Member State will vary depending on the potential for invasive introduction and spread of the species group. [...] An estimate of €30,000 with consumables (€24,000 personnel and travel + €6,000 lab consumables) was given by Dr. Marlen I. Vasquez, Cyprus University of Technology (Pers. Comm.), assuming there is already an operational lab and there is no need for new equipment. This refers to six months development, 12 months sampling campaign and six months for analysis. Setting up the lab (PCR equipment etc) would cost a minimum of € 20,000. The method requires the collection of water samples (1 to 10 L of water) from strategically placed sampling sites to search for the targeted species. These costs are lower if a lab is already doing similar routine work." Citizen science might also be used to detect new introductions (Delaney et al. 2008) and should be cost-free using existing technologies.	
Methods to achieve eradication	**Eradication**	If only a few few individuals arrived, particularly in winter when the species is not reproducing, containment and eradication might be possible. There are successful examples in Europe of eradication of freshwater fishes, mostly through rotenone (e.g. Britton et al. 2008); however, this is costly and probably often not feasible in the open habitats typical of this species (estuaries, coastal lagoons and salt marshes). After the recent introduction of mummichog in the Ebro delta in 2005, eradication with rotenone in the ditches where it was present was attempted in 2006. Although the eradication was apparently succesful	Medium

		(758 mummichog captured), the species was detected again in 2007 and was apparently already established in more open areas (Pou i Rovira 2008). Even in enclosed water bodies the use of rotenone brings significant environmental effects, consequences for other species and issues of public acceptability. Moreover, *Fundulus heteroclitus* often inhabits coastal wetlands of high conservation value, making eradication more difficult. Rotenone eradication costs "approximately £2 per m^2 of water area treated" (Britton et al. 2008, Oreska & Aldridge 2011). Oreska & Aldridge (2011) report the costs for many erradications with rotenone in the UK, mostly of the cyprinid *Pseudorasbora parva*. Rytwinski et al. (2018) recently reviewed non-native fish removal attempts in freshwater ecosystems and of those with adequate information, chemical treatments were relatively successful (antimycin 75%; rotenone 89%) compared to other interventions; electrofishing and passive removal measure studies indicated successful eradication was possible (58% each respectively) but required intensive effort and multiple treatments over a number of years. If mummichog arrived in an enclosed or isolated water body such as a ditch, channel or a small pond, nets to prevent spread and draining the channel or pond would be a possible eradication tool. The costs should be similar than for *Gambusia* spp., which were "roughly estimated to be medium to high (>€50k/ha)" by Verreycken & Copp in 2017 (Roy et al. 2018).	
Methods to achieve management	**Reducing risks of further dispersal**	If established in an area of a Member State, the methods mentioned above, mainly improving awareness, managing pathways and methods for eradication, would also be useful to support population control and reducing further spread. Rytwinski et al. (2018) recently reviewed non-native fish removal attempts in freshwater ecosystems and of those with adequate information, electrofishing had the highest success for population size control (56% of data sets). However, electrofishing will not be useful in the saline environments typical of mummichog and active (e.g. seines) or passive (minnow traps, fyke nets, etc.) methods will have to be used and can be expected to be less efficient. In the USA, the control	Medium

		costs of sea lamprey through lampricide for larvae control, barriers, traps, and a sterile male release program range from US $304,000 for New York to $3.3 million for Michigan (Lovell et al. 2006). A proposed 11-year ruffe (*Gymnocephalus cernua*) control program in the Great Lakes, through toxins, trawling, and ballast water management was estimated to cost US $13.6 million (Leigh 1998). As a further example, a recent review (Oreska & Aldridge 2011) estimates that the Great Britain-wide cost of controlling freshwater invasive species is approximately £26.5 million year-1 (as 2009 GBP) and could total £43.5 million year-1 if management efforts were undertaken at all infested locations; the former figure includes £15.9 million for site consultation, monitoring, and control of the fish *Pseudorasbora parva*.	

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2[1]

Name of organism: white perch *Morone americana* (Gmelin, 1789)

Author(s) of the assessment: Luke Aislabie, Hugo Verreycken, Daniel Chapman and Gordon H. Copp

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

Peer review 1: Felipe Ribeiro, University of Lisbon, Lisbon, Portugal
Peer review 2: Wolfgang Rabitsch, Umweltbundesamt, Vienna, Austria

This risk assessment has been peer-reviewed by two independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study.

Date of completion: 24 October 2018

[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

Contents

RISK SUMMARIES

	RESPONSE	CONFIDENCE[2]	COMMENT
Summarise Entry[3]	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	There exists the possibility of *M. americana* being introduced to, and establishing in the RA area. The TRANSPORT – STOAWAY pathway (ballast water) is the most likely way for *M. americana* to enter the EU. But despite the large number of daily shipping transports between the native range and Europe no single *M. americana* was ever recorded in the RA area even although most of the RA area is suitable habitat in current conditions. Deliberate introduction (e.g. for aquaculture or angling) is less likely as countries are unlikely to be interested in this species because they have native fish species of equivalent or higher commercial interest. Similarly, there are other fish species native to Europe that can be imported more easily from other EU countries than would be the transport of *M. americana* to Europe from North America.
Summarise Establishment[4]	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	*M. americana* have been shown to have the ability to inhabit a wide range of aquatic environments throughout its native and introduced ranges in North America. The comparison of Köppen-Geiger climate types (Peel et al., 2007) and the habitat suitability (invasibility) modelling undertaken for this RA (see Figures 3–5 here above) indicate that the RA area currently

[2] In a scale of low / medium / high, see Annex III
[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I
[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

			possesses suitable climate conditions for establishment of *M. americana*. In view of these factors, the species is likely to establish self-sustaining populations in the RA area if introduced under both current and future climate conditions.
Summarise Spread[5]	very slowly slowly **moderately** rapidly very rapidly	low **medium** high	*M. americana* is a semi-anadromous fish, which reduces slightly its ability to migrate from one river estuary to another. However, elevated precipitation on land results in elevated river discharges, which leads to a much wider dilution of coastal marine waters (in terms of salinity), and during such events, it is likely that *M. americana* could migrate between river estuaries of close proximity due to the reduced-salinity bridge created during concurrent high discharge events in the two neighbouring river estuaries. Equally, should the species be imported and become established, the risk of human-aided dispersal would increase, given the propensity of humans to translocate and release fish species for a wide variety of reasons, including angling amenity (Copp et al., 2005, 2007, 2010; Britton & Davies, 2006).
Summarise Impact[6]	minimal minor **moderate** major massive	**low** medium high	The literature evidence for the species' introduced range in North America (e.g. the Great Lakes) suggests that it can exert both competitive and predatory pressures on native fish species, but the extent of adverse impacts on other taxonomic groups, either directly (e.g. non-fish prey during early ontogeny) or indirectly (i.e. food web linkages) remains largely unstudied even in North

[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly
[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

			America. However, in absence of direct evidence of native species extirpation due to *M. americana* introductions, the likely impact of this species is currently estimated to be moderate, but with a caveat of low confidence.
Conclusion of the risk assessment[7]	low **moderate** high	low **medium** high	Overall, the range of risk responses and there is a generally low-to-moderate level of confidence associated with some aspects of the risk assessment. For this species, the overally risk, if it gains entry to the RA area is considered to be moderate, and that is with an overall moderate level of confidence. Whereas, escapee specimens of the Morone hybrid (*M. saxatilis* x *M. chrysops*) are known to persist in water courses of some EU countries (e.g. Safner et al., 2013; Skorić et al., 2013), and apparently has the capacity to spawn in Continental European climate conditions (Müller-Belecke et al., 2014, 2016). This suggests that the indicated moderate risk level for *M. americana* is appropriate. Given this information, as well as information acquired (during this RA) that refer to impacts of the three parent *Morone* species in their introduced North American ranges, it is recommended that a risk assessment be carried out for the EU on the *Morone* hybrid (*M. saxatilis* x *M. chrysops*).

[7] In a scale of low / moderate / high

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established (future)	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)	Established (future)	Invasive (currently)
Alpine	–	–	?	–
Atlantic	–	–	?	–
Black Sea	–	–	?	–
Boreal	–	–	?	–
Continental	–	–	?	–
Mediterranean	–	–	?	–
Pannonian	–	–	Yes	–
Steppic	–	–	Yes	–

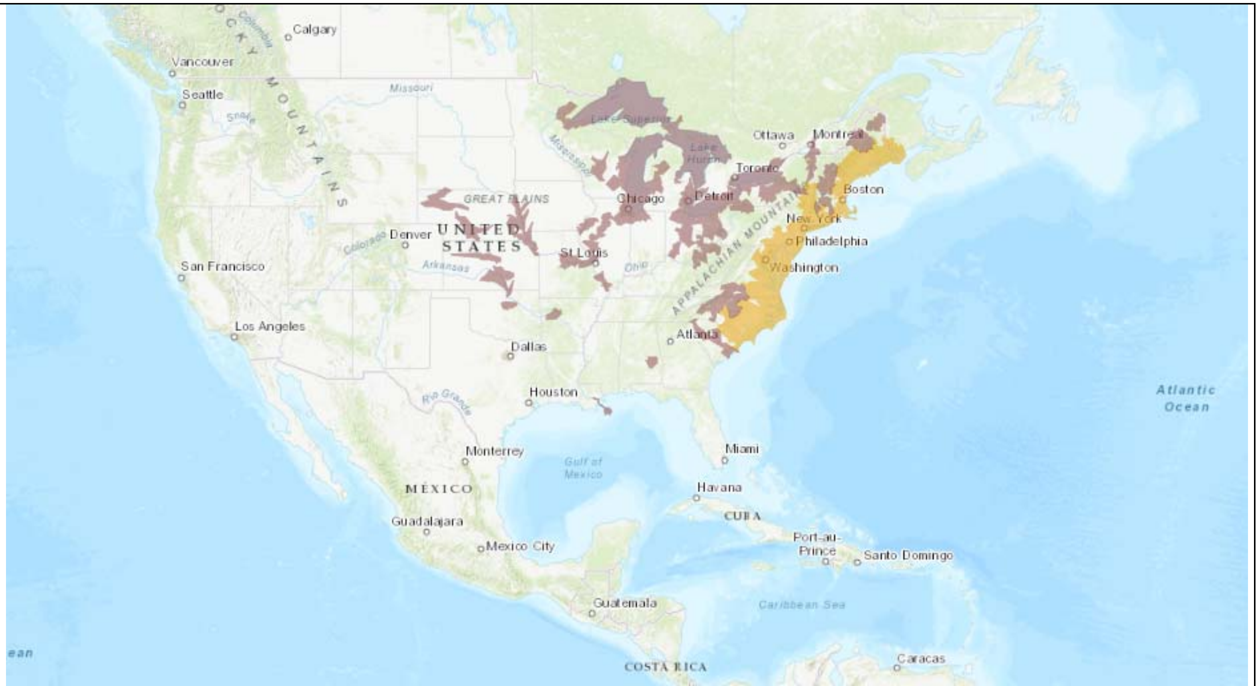
Marine regions and subregions of the risk assessment area

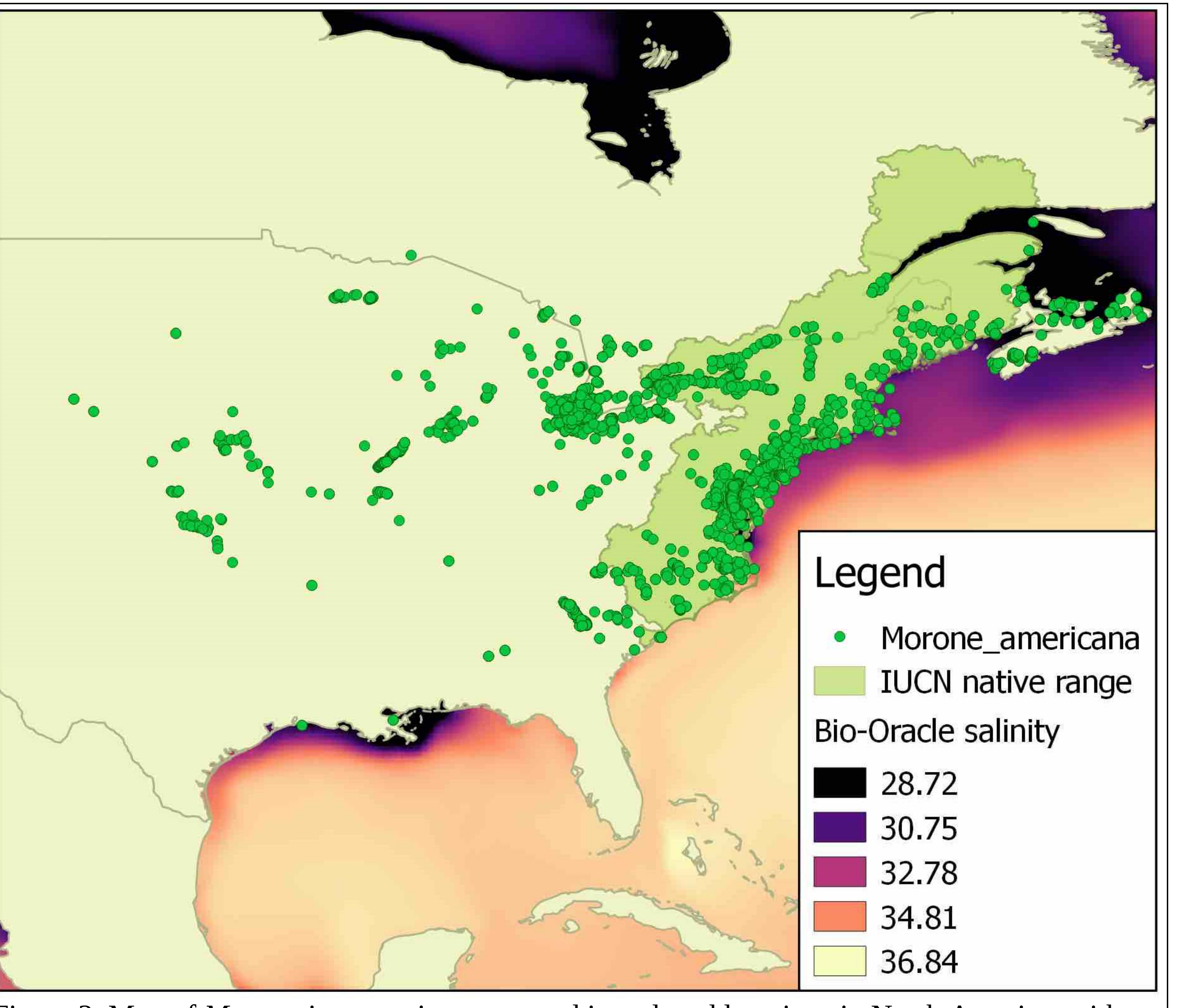
	Recorded	Established (currently)	Established (future)	Invasive (currently)
Baltic Sea	–	–	–	–
Black Sea	–	–	–	–
North-east Atlantic Ocean	–	–	–	–
Bay of Biscay and the Iberian Coast	–	–	–	–
Celtic Sea	–	–	–	–
Greater North Sea	–	–	–	–
Mediterranean Sea	–	–	–	–

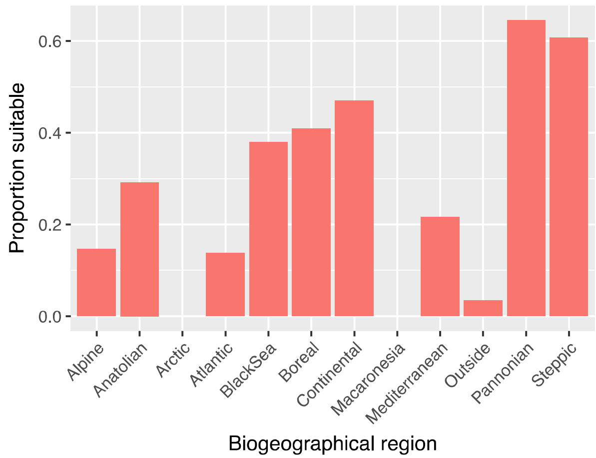
Adriatic Sea	–	–	–	–
Aegean-Levantine Sea	–	–	–	–
Ionian Sea and the Central Mediterranean Sea	–	–	–	–
Western Mediterranean Sea	–	–	–	–

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Domain: Eukaryota Kingdom: Metazoa Phylum: Chordata Subphylum: Vertebrata Class: Actinopterygii Order: Perciformes Suborder: Percoidei Family: Moronidae Genus: *Morone* Species: *Morone americana* (Gmelin, 1789) Common name: White Perch International common names: English: narrow-mouthed bass; sea perch; silver perch; wreckfish Spanish: lubina blanca French: bar blanc d'Amerique; baret; cernier atlantique; perche blanche Russian: morona Synonym: *Perca americana* Gmelin, 1789 Hybrids: *M. americana* · *M. chrysops* (Not included in this assessment; there is little information in the literature on this hybrid, which appears to be a less-successful hybrid than that of *M. saxatilis* · *M. chrysops*) Congener species: *M. saxatilis, M. chrysops, M. mississippiensis*
A2. Provide information on the existence of other species that look very similar [that may	The only other organism that is likely to look very similar to *M. americana* is the *Morone* hybrid (*M. chrysops* · *M. saxatilis*), which has been imported to some EU and neighbouring countries

be detected in the risk assessment area, either in the wild, in confinement or associated with a pathway of introduction]	for aquaculture, and there are a few reports of specimens of this hybrid being captured from EU rivers (Safner et al., 2013; Skorić et al., 2013; Kizak & Güner, 2014).
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	No this is the first formal risk assessment known to have been undertaken on this species by the authors.
A4. Where is the organism native?	Sea areas: Atlantic, Northwest Atlantic, Western Central North America: Canada: New Brunswick Nova Scotia Prince Edward Island Quebec USA: Connecticut Maryland New Jersey Rhode Island New Jersey Delaware Maryland Virginia North Carolina South Carolina (Froese & Pauly, 2004) (Fuller et al., 2006) (Able & Fahay, 2010)

A5. What is the global non-native distribution of the organism outside the risk assessment area?	 Figure 1 Map showing the native (beige) and non-native (mauve) distributions of white perch *Morone americana* in North America (USGS, 2018). Use of map copy permitted as per USGS Information Policies and Instructions: www.usgs.gov/information-policies-and-instructions/crediting-usgs).

	 Figure 2. Map of *M. americana* native range and introduced locations in North America, with the salinity of relevant marine areas indicated (see Annex VI).
A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment	None, however hybrids of two *Morone* species (*Morone chrysops* · *M. saxatilis*) has been reported in open waters of Croatia (Safner et al., 2013), Serbia (Skorić et al., 2013) and Turkey

area has the species been recorded and where is it established?	(Kizak & Güner, 2014), and the risk of reproduction of these hybrids in Germany has recently been examined which was deemed to be elevated (Müller-Belecke et al., 2014, 2016).
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 regions that span the EU projected to be suitable under current climate are examined in greater detail in the Q1.13, but in summary see Figure 3.  Figure 3. Proportion of projected suitable habitats within the RA area for *M. americana* by region in Europe (see Annex VI). For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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.	None of the EU member states have been recorded to have established populations of *M. americana*.
A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	Current climate: Most EU member states, possibly including northern parts of Sweden and Finland, but freshwater climate data were not available for the northern parts of those countries so .

	Future climate: All EU member states because they have been reported to be able to spawn between 10–16°C and in brackish (< 4 ppt) to freshwaters, which is sufficient for reproduction under current climate conditions except for two countries whereas in the future its possible they would be able to establish in all countries. (Mansueti, 1961; Jenkins and Burkhead, 1994; Able and Fahay 2010).
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?	*M. americana* is classified as invasive in some parts of the USA and Canada (Cooke, 1984; Boileau, 1985; Harris, 2006; Kuklinski, 2007; Cavaliere et al., 2010), and has been listed amongst invasive species recorded in about five protected areas of the south Atlantic area of North America (Benson et al., 2016). Example of this is shown in Q 1.26.
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	None
A12. In which EU member states has the species shown signs of invasiveness?	None
A13. Describe any known socio-economic benefits of the organism.	*M. americana* is used as a food source for humans (Wisconsin Sea Grant, 2002) and is considered to be a popular sport fish throughout the native range in North America, where recreational angling for them for consumption is known to occur in the Mid-Atlantic states. There is commercial fishing of the species, using trawls, haul seines and drift gill nets, in some areas, with Chesapeake Bay (USA) being the most popular (Ballinger & Peters, 1978; Etnier & Starnes. 1993; Animal Diversity Web, 2018; Page & Burr, 1991).

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.”
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	**RESPONSE** [chose one entry, delete all others]	**CONFIDENCE** [chose one entry, delete all others]	**COMMENT**
1.1. How many active pathways are relevant to the potential introduction of this organism?	none **very few** few moderate number	low **medium** high	*M. americana* is not present in the risk assessment (RA) area. Expansions from the NE coast of the USA further west occurred mainly by natural migration via canals. Other pathways described by

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

[9] 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 respond N/A and move to the Establishment section)	many very many		Fuller et al. (2008) are accidental introduction of young of the year, produced in a hatchery, into a reservoir, intentional stocking for sportfishing, stock contamination from a striped bass stocking, illegal stocking and via ships' ballast water. Only the last pathway can possibly be an active pathway of introduction into the RA area. There is no evidence of introduction of white bass (eggs, larvae, …) for aquaculture in the EU (Froese and Pauly, 2018).
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	a. TRANSPORT - STOWAWAY (Ship/boat ballast water) b. TRANSPORT – CONTAMINANT Contaminant on animals i.e. for aquaculture		a). There are huge transports of ballast water between the native range of *M. americana* (East USA) to the RA area. However, up till now, no populations or even specimens of *M. americana* have been reported for Europe. New stricter regulations for ballast water treatment are in force since 2017 (Ballast Water Convention) so the potential of introduction via ballast water would be further limited. b). *Morone* species, including *M. americana* (Hushak et al., 1993), are of aquaculture interest, and a hybrid of two *Morone* species has been imported to some EU and neighbouring countries (e.g. Israel) for aquaculture (Nelson, 1994), i.e. *Morone saxatilis* · *M. chrysops*, with specimens having been reported in open waters in Croatia (Safner et al., 2013), Serbia (Skorić et al., 2013) and Turkey (Kizak & Güner, 2014). This hybrid seems to be considered as an attractive game fish in Italy, Germany and Turkey (Roncarati et al., 2009; Müller-Belecke et al., 2016). *M. americana* may be a stowaway in aquaculture transports of hybrid *Morone*.

	c. RELEASE IN NATURE (Fishery in the wild)		In the USA, *M. americana* have been stocked intentionally in non-native waters by voluntary and incidental agency stocking, and possibly by angler introductions in other areas for sport fishing (CABI, 2018). Intentional stocking of *M. americana* in the RA area should not be possible or should be well regulated as it concerns an alien species (under the EU Regulation on the Use of Alien Species in Aquaculture; European Council 2007) but illegal stocking by individual anglers for sport fishing would be hard to prevent. Of course, the anglers would first have to be able to obtain a sufficient number of *M. americana* specimens, transport them between North American and Europe, which would be difficult to do with low mortality rates.
Pathway name:	TRANSPORT - STOWAWAY (Ship/boat ballast water)		
1.3a. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional **unintentional**	low medium **high**	
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Although there are huge transports of ballast water between the native range of *M. americana* (East USA) to the RA area, the chance for *M. americana* to be taken in ballast water tanks in large numbers seems small since *M. americana* spawn in shallow waters and the eggs sink to the bottom. Despite the daily shipping transport between native range and Europe no single *M. americana* was ever recorded in the RA area.

1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	Survival of eggs or young-of-the-year fish in ballast water tanks is likely to be low-to-moderate due to ballast water treatment (e.g. filters, UV radiation) and other sub-optimal conditions like low dissolved oxygen, etc. as well as shear stress in relatively confined spaces (Morgan et al., 1979). Also, the exchange of ballast water from fresh/brackish to sea water (if applied) will be detrimental to young-of-the-year *M. americana*. Reproduction will not occur since adult specimens are unlikely to survive being taken up via ballast water pumps.
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	See Q1.5
1.7a. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	If *M. americana* would arrive by ballast water, then it would go entirely unnoticed until larger specimens would be found in the receiving waters, this happened to many aquatic species before (e.g. in the Laurentian Great Lakes (USA), Vander Zanden et al., 2010).
1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	Extensive daily transports occur between the native range of *M. americana* and the RA area, so this would also cover the most appropriate time of the year for establishment.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely likely **very likely**	low medium **high**	The organism would be transferred straight from the ballast water into the receiving waters of the main European ports, which are situated in estuaries where circumstances suitable to the species exist, mainly brackish water (North & Houde, 2003).
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely **unlikely**	low **medium**	In absence of detailed information on ballast water exchanges between North America and the RA

	moderately likely likely very likely	high	area, it is difficult to predict whether or not *M. americana* could be introduced via this pathway. However, locations where ballast water could be taken on in the native range could contain small *M. americana*, but their survival through the pumps and during the trans-Atlantic voyage would seem to be unlikely – otherwise, the species would have most likely been reported from somewhere in the RA area.
Pathway name:	TRANSPORT – CONTAMINANT (Contaminant on animals e.g. for aquaculture or stocking)		
1.3b. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional **unintentional**	low medium **high**	The organism can be a contaminant of imported fish for aquaculture/stocking. The source of *M. americana* in two Kansas reservoirs is a result of stock contamination from a striped bass stocking (Fuller et al., 2018).
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	Production of *Morone* hybrids in Europe is limited to Italy, Portugal, France, Germany, Italy, with the nearest non-EU state being Israel (Gottschalk et al., 2005; FAO, 2018) and information on the import of *Morone* species or hybrids to the RA area were not accessible. Also stocking with *Morone* species in the EU is undocumented with *M. americana* infested transports of other *Morone* species in large numbers from the native area to Europe therefore seem unlikely.

1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	If live transport of *Morone* species were to be organised, then survival during the passage would be high as with other fish transports. Reproduction during the transport is very unlikely.
1.6b. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	As the introduction of other *Morone* species for aquaculture is intentional, no management practices will be employed to kill the animals. Therefore, *M. americana* would be likely to survive in the absence of management practices. .
1.7b. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	In the unlikely event of *M. americana*, a species not the subject of aquaculture, to find its way into an aquaculture facility that rears the hybrid *M. chrysops* · *M. saxatilis*, then it is likely that *M. americana* would go undetected in consignments of the above-mentioned hybrid from the USA to the RA area, especially if the consignments were those of eggs or fry.
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Live transports of *Morone* species for aquaculture could be organised at any time of the year.
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Successful incidental escape from an aquaculture facility may happen, which is likely to be within the vicinity of a water course and its estuary, where circumstances suitable to the species exist, mainly brackish water (North & Houde, 2003). The occurrences of *Morone* hybrids in the Danube attest this possibility (Safner et al. 2013; Skorić et al. 2013).
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely **unlikely**	low **medium**	Since there is limited use of this species in aquaculture in its native range, and no apparent

	moderately likely likely very likely	high	link with non-native species imported from the native range and aquaculture in the RA area, importation as a contaminant is unlikely.
Pathway name:	RELEASE IN NATURE – Fishery in the wild		
1.3c. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	**intentional** unintentional	low medium **high**	
1.4c. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	*M. americana* are being illegally stocked for sport fishing in inland lakes in Indiana (Fuller et al., 2018). In some Member States of the EU, illegal stocking of non-native species for sport fishing has happened (or still is happening) e.g. asp *Aspius aspius* in the River Meuse in the Netherlands and Belgium (Verreycken et al., 2007) (and probably many more). This could also happen with *M. americana* provided a sufficient number of specimens would be available in the RA area. However, except for direct import from North America, these fish would be very hard to get in sufficient numbers to originate a viable population.
1.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	*Morone* species e.g. *M. saxatilis* have a high tolerance for environmental stress such as elevated temperature (28°C) or hypoxia (3 mg/L O2) although a combination of stress factors will affect their metabolic performance (Lapointe et al., 2014). It can thus be assumed that *M. americana* can survive transport and stocking, especially since people who would perform the stocking would try

			to keep the environmental factors during transport as optimal as possible. Reproduction during the introduction would be very unlikely since suitable habitat is missing.
1.6c. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	As the introduction of other *Morone* species for angling is intentional, no management practices will be employed to kill the animals. Therefore, *M. americana* would be likely to survive in the absence of management practices. It would, however, be easy to kill *M. americana* with piscicides. But tracing and locating illegal transport and stocking would be difficult.
1.7c. How likely is the organism to enter the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	It will be difficult to trace and halt illegal stocking of fishes. Although many MSs have fish monitoring programmes, it could take several years before *M. americana* was noticed, depending upon the monitoring systems and public awareness at the national, regional and local levels.
1.8c. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Live transports of *Morone* species for stocking could be organised at any time of the year.
1.9c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Intentional stocking of fish species, e.g. for angling purposes, would be expected to be transferred to receiving waters that are suitable habitat for the species. Many of the European waters seem to be suitable habitat for *M. americana* (see Figure 3).
1.10c. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	Although illegal stocking of fishes for angling purposes is an on-going problem (e.g. Aps et al., 2004; Copp et al., 2010), illegal stocking of *M. americana* in the RA area will be limited and thus the likelihood of entry via this pathway unlikely.

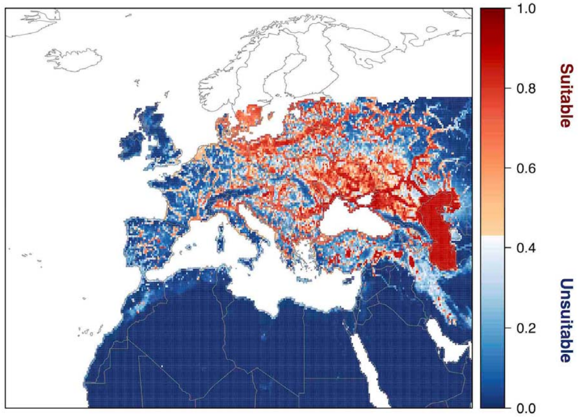
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	very unlikely **unlikely** moderately likely likely very likely	low **medium** high	Of all of the above-mentioned pathways, the TRANSPORT – STOAWAY pathway is the most likely way for *M. americana* to enter the EU. But despite the large number of daily shipping transports between the native range and Europe no single *M. americana* was ever recorded in the RA area even although most of the EU is suitable habitat in current conditions.
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Of all of the above-mentioned pathways, the TRANSPORT – STOAWAY pathway is the most likely way for *M. americana* to enter the EU. But despite the large number of daily shipping transports between the native range and Europe no single *M. americana* was ever recorded in the RA area. However, trade may get more intense in the future thus increasing the possibility of entry and, on top of that, climate warming would slightly enlarge the number of MSs where suitable habitat would be available. Therefore, the overall likelihood of entry into the RA area based on all pathways in foreseeable climate change conditions is estimated as moderately likely.

PROBABILITY OF ESTABLISHMENT

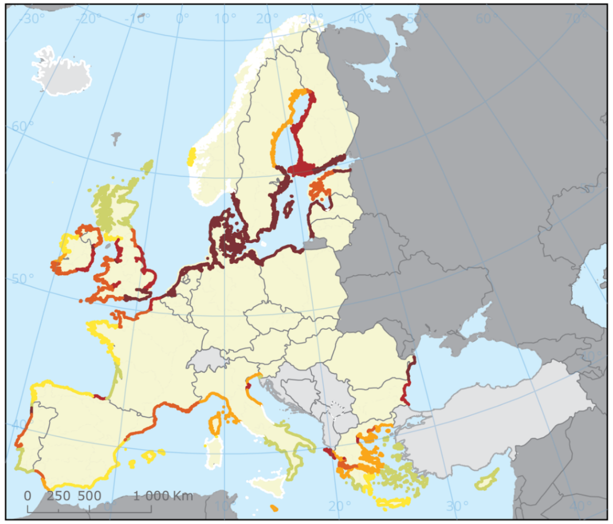
Important instructions:

- 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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely **likely** very likely	Low **medium** high	Comparison of the species' current native and introduced ranges in North America in terms of Köppen-Geiger climate type (Peel et. al., 2007) suggest largely similar climatic conditions to the RA area, and this is further supported by GIS-generated map overlays (Figure 4), with parts of Central Europe (Pannonian and Steppic regions) projected to be particularly suitable. Not included in these overlays are salinity levels and the presence of water retention structures, which are well-known barriers to migration (Ovidio & Philippart, 2002). Further uncertainty in these projections arises from the fact that the species has not yet been observed invading outside North America, where it has a strong association with major river systems. Based on the species mostly occupying major river systems in North America, the model identified large rivers as the main limiting factor in Europe, but if the species is able to invade smaller water courses in Europe, then the suitable region could be larger than estimated in Figure 4.

			 Figure 4. Map of projected suitable habitat for *M. americana* in the RA area (see Annex VI) – See also Figure 3 for the proportions of projected suitable habitat by biogeographic region within the RA area. The most compelling evidence available for *M. americana* establishment risk comes from Germany (Müller-Belecke et al., 2014, 2016), where a recent study reported successful spawning of the *Morone* hybrid (*M. saxatilis* x *M. chrysops*) in static outdoor water tanks without hormonal treatment, followed by the collection of hundreds of "hatched larvae". This strongly suggests, given the lentic condition of the outdoor tanks and the similar climate range and environmental biology of the parent species of the hybrid (Fuller 2018; Fuller & Neilson, 2018), that natural reproduction of other *Morone* species, such as *M. americana*, is likely.

1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	very unlikely unlikely moderately likely likely **very likely**	low **medium** high	The abiotic conditions in its current distribution are similar to the RA area and there are no obvious differences between the two to indicate that establishment would not be likely in the risk assessment area.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	very isolated isolated Moderately widespread **widespread** ubiquitous	low medium **high**	The species occurs in fresh, brackish and coastal waters. Usually found in brackish waters or close to shore, however it can be found in rivers or ponds usually over muddy substratum. (Able & Fahay, 2010; Cabi, 2018). Transitional waters, which offer conditions suitable to the species (North & Houde, 2003; Able & Fahay, 2010), are abundant throughout the RA area, suggesting an elevated likelihood of establishment throughout the region. (See also response to Q1.13). All EU countries except Hungary, Slovakia, Austria, Luxembourg and the Czech Republic, i.e. 82% of the EU, possess transitional waters (Figure 5), with coastal and estuary habitat representing 45 000 km^2 of EU territory (European Council 1992: Pariona, 2018). This suggests the species would find suitable habitat

			(see also Figures 3 and 4) throughout most of the RA area.  **Figure 5. Map indicating the coastal and transitional waters across Europe (EEA, 2018).** (Use of map copy permitted as per EEA Copyright Notice: www.eea.europa.eu/legal/copyright).
1.16. 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?	**N/A** very unlikely unlikely moderately likely likely very likely	low medium **high**	There is no evidence to suggest, and it is unlikely that, this species requires another species to complete its lifecycle
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	The species has been shown to successfully compete, and in some cases outcompete other species. Based on examples from locations in North America, such as the US state of Indiana and the Great Lakes (e.g. Michigan) where the species has been translocated, it is

			likely that *M. americana* could establish within the RA area irrespective of competition from native species (Encyclopedia of Life, 2018; Schaeffer & Margraf, 1986). Moreover, being a species with high temperature and salinity range limits (Able & Fahay, 2010), this specie might circumvent any competition effect by occupying different habitats .
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	The only known predator in the RA area is the northern pike (*Esox lucius*), although it has been known to be eaten by walleye (*Sander vitreus*), which has at least two congeners in Europe that could exert similar predation pressure (biological resistance) (Ward and Neumann, 1998): pikeperch (*Sander lucioperca*), and Volga pikeperch (*Sander volgensis*). Another potential predator is the European catfish (*Silurus glanis*), which is known to predate on a wide range of fish species (Copp et al., 2009). However, there are relatively few cases of biological resistance amongst large-bodied fishes, and no such biological resistance has been evidenced for the species introduced range in North America where at least as many potential predators exist than the RA area, so it is unlikely predators would impede establishment. *Kudoa* sp. is a known parasite infecting this *M. americana*, being present in other fish in RA (Buton & Poyton, 1991; Yurakhno et al., 2007), but no information about its potential impact in the RA was found.

1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very unlikely unlikely moderately likely **likely** very likely	**low** medium high	Given that the species has successfully established in parts of the USA and Canada which are outside of the native range, this would indicate that *M. americana* could establish within the RA area dependent on where they are introduced. Another factor to consider is there are a range of non-native species that have established within the EU such as top-mouth gudgeon and pumpkinseed sunfish which would suggest that under current management practices this is unlikely to affect establishment of this species (Leppäkoski et al., 2011).
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	Existing management practices for brackish waters and coastal areas are very limited so this would help to facilitate establishment of this species as there would be very little disturbance to the habitat except for commercial fishing vessels trawling. In relation to lowland water courses, there is no information to suggest that it would affect *M. americana* from establishing.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	very unlikely unlikely moderately likely likely **very likely**	low **medium** high	*M. americana* inhabit coastal and transitional waters which would suggest that any eradication campaign would be likely to be unsuccessful due to the ability of the species to inhabit a range of habitats and they are predominately found to be in brackish waters (estuaries) and it is not possible to isolate the water body, it would be impossible for all the species to be eradicated (Williams & Grosholz, 2008). If they were to be introduced in to lakes or rivers that do not discharge into the sea then it is likely that eradication could be possible.

			However, if the river does discharge into the sea then this would again likely prevent the successful eradication of the population.
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	very unlikely unlikely moderately likely likely **very likely**	low **medium** high	*M. americana* are known to spawn in fresh waters in temperatures of between 10–16°C, but spawning has been shown in temperatures up to ≈20°C (Mansueti, 1961; Jenkins and Burkhead, 1994; Able and Fahay, 2010). The species does not show a preference with regard to habitat type during spawning and egg deposition (Zuerlein, 1981), however, there is evidence of specific parts of rivers being selected for spawning (Kraus & Secor, 2004). Optimal nursery conditions are believed to involve turbid (food rich) brackish areas with low salinities, which are predicted to be influenced by river discharge (North & Houde, 2003). This suggests that the species could spawn in a range of different countries within the RA area if they were to be introduced into suitable open waters.
1.23. How likely is the adaptability of the organism to facilitate its establishment?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	The adaptability of the species has received limited research however, there is some information on habitat preferences, e.g. temperature (Hall et al., 1979), and it has been shown that when it has been introduced into a water body, it can establish if the food source and water quality is within its parameters (Johnson & Evans, 1990). Laboratory experiments provided evidence that "differences in overwinter behaviour, metabolism, and survival appear to be adequate to account for observed differences in survival of these species in the wild"

			(Johnson & Evans, 1991). *Morone* species e.g. *M. saxatilis* have a high tolerance for environmental stress such as elevated temperature (28°C) or hypoxia (3 mg/L O_2) although a combination of stress factors will affect their metabolic performance (Lapointe et al., 2014). Moreover, considering both the latitudinal range in the native area and the different occupied habitats, *M. americana* is highly like to exhibit some degree of adaptability in the RA (Able & Fahay, 2010).
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	Although no research has been carried out on this, it is possible to come to the assumption that due to this species prolific reproduction, the species is very likely to establish with a low genetic diversity in the founder population (Jenkins & Burkhead, 1994).
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	This species is known to be established within large parts of The USA and Canada (CABI, 2018). This question is partially answered in Q1.13 in relation to the similarities in climate conditions. Bethke et al. (2014) reported through various sources that *M. americana* are "excellent competitors and invaders due to a variety of life history traits…", which emphasises that it is likely they would be able to establish within the RA area.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	**very unlikely** unlikely moderately likely likely very likely	low **medium** high	It is unlikely that a casual population will be possible to continue to occur because as records shows, there is no indication that the species is kept anywhere within the RA area meaning that it's not possible for continual release or any similar methods. In Indiana (USA), where the species is classified as invasive, there are laws

			that force anglers or someone that finds the species to kill them and they could be prosecuted if released alive (State of Indiana, 2005).
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	very unlikely unlikely moderately likely likely **very likely**	low **medium** high	*M. americana* can tolerate a range of water quality parameters such as salinity tolerances and water temperature etc. which would allow establishment in a range of locations in current conditions located within the Pannonian and Steppic biogeographic region as well as the Continental, Boreal and Black Sea regions (see Figure 3). Although the species is not in the RA area yet, it is possible to assume due to the parameters it can withstand, that if the species was to get to the area through abovementioned pathways, then it is very likely they could establish.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	With the increase in water temperatures forecasted through climate change, this would suggest that more locations within the risk assessment area will become more accessible for *M. americana* especially in north and central Europe as well as parts of the Mediterranean and Atlantic biogeographical regions (Lindner et al., 2010; Baki, 2018). Although it is hard to give definitive answers on how much temperatures will increase, it has been shown that it is currently on a rising trend and no evidence to prove otherwise (www.GlobalChange.gov, 2018).

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	minimal minor **moderate** major massive	low **medium** high	In North America, *M. americana* is known to have actively migrated from its native range to the Great Lakes region through canals and waterways between drainage basins. The introduction and spread of *M. americana* in the USA is detailed in Fuller et al. (2008). If this species were to be introduced in the RA area, then it could spread easily through watersheds because of the many connections between them. The temperate climate in most of the area would fit perfectly for the *M. americana*. As *M. americana* is an estuarine species with a broad salinity range (Natureserve, 2008; Able & Fahay, 2010), it probably can find suitable habitats easily. It is possible that natural disasters such as flooding could provide an opportunity for *M. americana* to spread across water bodies and through rivers (Jackson et al., 2001). However, *M. americana* have been classified as a partial migratory species. It has been known to migrate from fresh to brackish waters or coming

			in from the sea to freshwater to spawn. However, no research has shown that they have migrated across the sea which could limit their distribution (Kerr & Secor, 2009; Chapman et al., 2012). In fact, the population structure observed in the native range supports this (Mulligan & Chapman, 1989; Bian et al., 2016). For example, if they were found in the UK, it may be possible that they will not migrate to mainland Europe and establish a population. This would require human intervention for dispersal across a sea. All these dispersals are dependent on where the species is first (and subsequently) introduced in the RA area. The species is only semi-diadromous, which means spread from one river catchment to another would require a reduced-salinity 'bridge' between adjacent river estuaries in order to spread along a coastline.
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	minimal minor **moderate** major massive	low **medium** high	In the USA, *M. americana* have been stocked intentionally in non-native waters by voluntary and incidental agency stocking, and possibly by angler introductions in other areas for sport fishing (CABI, 2018). Under EU legislation, intentional importations of *M. americana* in the RA area would be regulated under Use of Alien Species in Aquaculture Regulation, and most likely limited to enclosed facilities. But, once in the EU, if unauthorised persons were able to access the enclosed facilities, then illegal stocking by individual anglers for sport fishing would be possible. This would seem unlikely due to the necessary security measures associated with enclosed aquaculture facilities.

			It is possible humans could introduce them as a means of sport fishing as they were in parts of The USA (Wisconsin Sea Grant, 2002b). Previously, it has been stocked into Kansas reservoirs accidentally as it got contaminated with a striped bass stocking (Fuller et al., 2018).
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	a. UNAIDED – NATURAL DISPERSAL		
Pathway name:	UNAIDED - NATURAL DISPERSAL		
2.3a. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	intentional **unintentional**	low medium **high**	
2.4a. 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?	very unlikely unlikely **moderately likely** likely very likely	low **medium** high	Introductions from the NE coast of the USA to water bodies further west mainly happened through active migration via canals (Fuller et al., 2018). If *M. americana* would arrive in large numbers in the RA area, e.g. via ballast water, then active migration would certainly be the main factor for spread. However, since only young life stages of *M. americana* (eggs, young-of-the-year) are expected to be introduced, viable populations will only be formed a few years after the

			introduction (males may spawn for the first time at age 2 years, and females usually by age 3 years).
2.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	The waters of the temperate part of the RA area would offer a suitable habitat for the spread and survival of *M. americana*, and also reproduction would certainly be possible along this pathway (cf. invasion history in the USA; CABI, 2018).
2.6a. How likely is the organism to survive existing management practices during spread?	very unlikely unlikely moderately likely **likely** very likely	low **medium** high	*M. americana* can easily be killed by rotenone (acute toxicity to *M. americana* was anticipated to be within recommended concentration levels on product label for similar fish and was corroborated by laboratory bioassay (LC_{100} of 0.15 mg/L Wujtewicz et al., 1997) or other piscicides. However, it would be difficult (if not impossible) to make an effective eradication in the lower course of rivers, especially large ones. Also, rotenone application is illegal in several EU member states.
2.7a. How likely is the organism to spread in the risk assessment area undetected?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	There exists no dedicated monitoring of invasive fish species in European rivers and canals, so once introduced, *M. americana* would be able to spread unnoticed until captured.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very unlikely unlikely moderately likely **likely** very likely	low medium **high**	The organism would be introduced from ballast water into the receiving waters of the main European ports where ideal circumstances exist (mainly brackish water) for survival of *M. americana*. Spread from there to suitable habitat will be easy.
2.9a. Estimate the potential rate of spread within the Union based on this pathway (please provide quantitative data where possible)	very unlikely unlikely **moderately likely** likely	low **medium** high	The potential for spread based on this pathway (CORRIDOR – INTERCONNECTED WATERWAYS) will depend on the success of the primary introduction and entry pathway

	very likely		(TRANSPORT -STOWAWAY (Ship/boat ballast water)). If several independent introductions (in different river basins) would occur then the overall spread would be greater than when it would with a single introduction.
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	very easy easy with some difficulty **difficult** very difficult	low **medium** high	Spread of *M. americana* in the RA area through 'CORRIDOR – Interconnected waterways' is currently non-existing (no records of *M. americana* in the area yet). However, would the species arrive in the area, it would be difficult to contain because natural dispersal is difficult to prevent.
2.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues and please provide quantitative data where possible).	very slowly **slowly** moderately rapidly very rapidly	**low** medium high	The potential for spread will depend on the number of introductions and the interconnectivity of the waterways. Overall spread risk would be greater in the case of several independent introductions (in different river basins) than in the case of a single introduction. *M. americana* is a semi-anadromous fish, which reduces slightly its ability to migrate from one river estuary to another. However, elevated precipitation on land results in elevated river discharges, which leads to a much wider dilution of coastal marine waters (in terms of salinity), and during such events, it is likely that *M. americana* could migrate between river estuaries of close proximity due to the reduced-salinity bridge created during concurrent high discharge events in the two neighbouring river estuaries. Still this would be uncommon events so spread though the RA area is likely to be slow.

2.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (please provide quantitative data where possible)	very slowly slowly **moderately** rapidly very rapidly	**low** medium high	Given the species' temperature tolerances (preferred mean temperature of coldest month >0°C and <18°C; mean warmest month >10°C (CABI, 2018)), climate change could potentially exert an influence on dispersal throughout most of the RA area. But see 2.11.

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is 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?	minimal **minor** moderate major massive	**low** medium high	There is evidence that *M. americana* have had adverse effects on biodiversity and ecosystems in various locations in The USA and Canada – see response to A4 (Allan & Zarull, 1995; Schaeffer & Margraf, 1987; CABI, 2018). For example, this species has been known to predate on fish eggs, adversely effecting on the recruitment of the predated fish populations (Schaeffer et al., 1987), e.g. in Lake Erie, predation on eggs of walleye (*Sander vitreus*), white bass (*Morone chrysops*) as well as cannibalism of their own eggs (Schaeffer et al., 1987). It remains unknown whether or not these reported cases of *M. americana* predation on native fish eggs have exerted an adverse effect on biodiversity.

2.14. 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)?	Not applicable	low medium **high**	Not applicable because the species does not occur, and has never occurred in the RA area.
2.15. 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?	minimal minor **moderate** major massive	**low** medium high	It is possible that the impacts will be similar to those stated in Q2.13 because the species has already been found to have these characteristics when previously invaded other areas and there is no evidence to suggest that this would be any different if found in the RA area.
2.16. 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?	Not applicable	low medium **high**	The species does not occur, and to our knowledge never occurred, in the RA area, so no impact could have been registered.
2.17. 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?	minimal minor **moderate** major massive	**low** medium high	If the species is found in the RA area, then it could potentially influence native species of conservation value with regard to European and national nature conservation legislation due to predation on eggs as seen in previous studies, although it has not been known to cause a major effect (Schaeffer et al., 1987). The Eurasian perch (*P. fluviatilis*) is virtually identical to *P. flavescens* (Thorpe, 1977), and there are likely to be other native species in the RA area, e.g. *Sander volgensis* (a threatened and protected species), that could also be adversely affected if *M. americana* were to be introduced and establish in the RA area
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	minimal **minor** moderate	**low** medium high	In its current non-native range, which does not include the RA area, *M. americana* is known to predate on the eggs of native fishes and to have the

	major massive		ability to out compete other species for food. For example, in Lake Erie, *M. americana* was found to have predated on walleye (*Sander vitreus*), white bass (*Morone chrysops*) as well as their own eggs (Schaeffer et al., 1987). These pressures could have an indirect, i.e. minor, impact on cultural services.
2.19. 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)?	Not applicable.	low medium **high**	The species does not occur, and to our knowledge never occurred, in the RA area, so no impact could have been registered.
2.20. 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?	minimal **minor** moderate major massive	**low** medium high	With climate change predictions from Q2.28, it provides evidence that establishment is possible within the RA area in the future and the answer to this question would be similar to the impacts in Q2.18. There is no evidence to say a different outcome would occur in the RA area. The main difference would be that this species would be predating and outcompeting different species although some species are very similar to species found within the RA area as stated in Q2.23.
Economic impacts			
2.21. 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	**minimal** minor moderate major massive	low medium **high**	In terms of costing, there is no evidence to give a monetary value on it but it has shown through previous questions that is has impacted other species which has had an effect on recreational angling. An example is explained in Q2.23.
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)?	Not applicable.	low medium **high**	The species does not occur, and to our knowledge never occurred, in the RA area, so no impact could have been registered.

*i.e. excluding costs of management			
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	minimal **minor** moderate major massive	low **medium** high	The possible negative impact of *Morone americana* on ecosystem services is caused predation on and competition with native species. *Morone americana* is considered to have had a moderate socio-economic impact in the Great Lakes of North America (Fuller et al., 2018): "The collapse of the walleye (*Sander vitreus*) fishery in the Bay of Quinte (on the north shore of Lake Ontario) coincided with an increase in the white perch population and may have been a result of egg predation and lack of recruitment (Schaeffer & Margraf, 1987). Other recreationally/commercially important species, such as white bass (*Morone chrysops*), yellow perch (*Perca flavescens*), and species of forage fish are likely negatively affected by white perch through competition, egg predation, or hybridization." The Eurasian perch (*P. fluviatilis*) is virtually identical to *P. flavescens* (Thorpe, 1977), and there are likely to be other native species in the RA area, e.g. *Sander volgensis* (a threatened and protected species), that could also be adversely affected if *M. americana* were to be introduced and establish in the RA area. The 'minor' response reflects the unlikelihood of *M. americana* being imported to EU countries due to current legislation in place to prevent this species entering the RA area.
2.24. 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)?	Not applicable.	low medium **high**	The species does not occur, and to our knowledge never occurred, in the RA area, so no impact could have been registered.

2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	minimal **minor** moderate major massive	low **medium** high	See response to Q2.23. Although there are no management costs in relation to the future, it is hard to give an estimate due to there being no cost estimates in relation to its current non-native range, which does not include the RA area.
Social and human health impacts			
2.26. 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).	**minimal** minor moderate major massive	low **medium** high	No direct information was found from the species non-native range outside of the RA area with regard to social, human health or other impact (not directly included in any earlier categories).
2.27. 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.	**minimal** minor moderate major massive	low medium **high**	With the species unlikely to established in the RA area in the future due to legislation put in place to prevent this however the response is similar to Q2. 26. Possible wider societal impacts could arise if the invasion has negative impacts on fisheries and other ecosystem services (see 2.23) and starts to threaten local livelihoods. However, there is no evidence to indicate major impacts of this type from the species' current introduced range, which does not include the RA area.
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	**minimal** minor moderate major massive	low **medium** high	No information was found on *M. americana* exerting damage to other organisms (other than predation, mentioned previously), however with any importation of non-native species from another continent, there is a risk of infectious agents being introduced. If *M. americana* were to be introduced for any aquaculture use, then it would fall under the EU Regulation on the use of alien species in aquaculture (European Council, 2007) for which a full risk analysis scheme has been developed, including an assessment module specifically on

			infectious agents (Copp et al., 2016). One parasite group mentioned as associated with *M. americana* is the myxosporean parasite genus *Kudoa* (Bunton & Poynton, 1991), and a review of this genus lists some European fish species of commercial and agriculture interest as being susceptible (Moran et al., 1999). The parasites and pathogens of this *M. americana* are likely to infect other Moronidae species native to RA (due to co-evolutionary history and phylogenetic relatedness), with some highly important in terms of fisheries management and aquaculture (eg. *Dicentrarchus labrax* – sea bass).
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	**NA** minimal minor moderate major massive	low medium high	None come to mind.
2.30. 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?	minimal **minor** moderate major massive	low **medium** high	There are reports that *M. americana* poses a problem for freshwater fisheries managers due to this species being excellent competitors and as previously said feeding on eggs of native species (Madenjian et al., 2000; Gosch et al., 2010). *M. americana* is likely to be a prey species to some European piscivorous species of fish and bird, but none is likely to exert a level of predation pressure that would result in *M. americana* extirpation should the species be introduced and establish itself in RA area waters.

ANNEXES

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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
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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 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.

[10] Not to be confused with „no impact“.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.*. 2017)

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[11]	**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)

[11] 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*

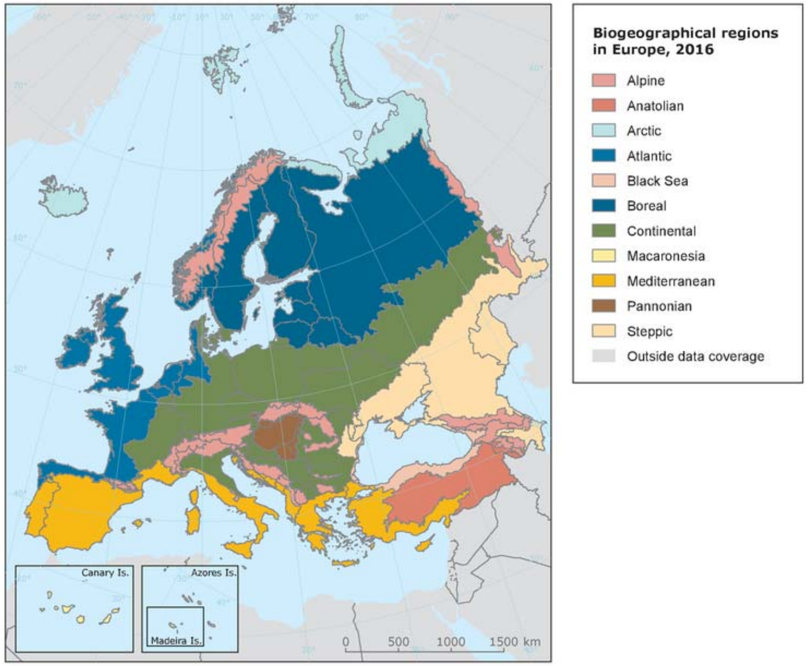
		Other biotic characteristics that have a **non-use value**	Characteristics or features of living systems that have an existence value; Characteristics or features of living systems that have an option or bequest value *Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.*

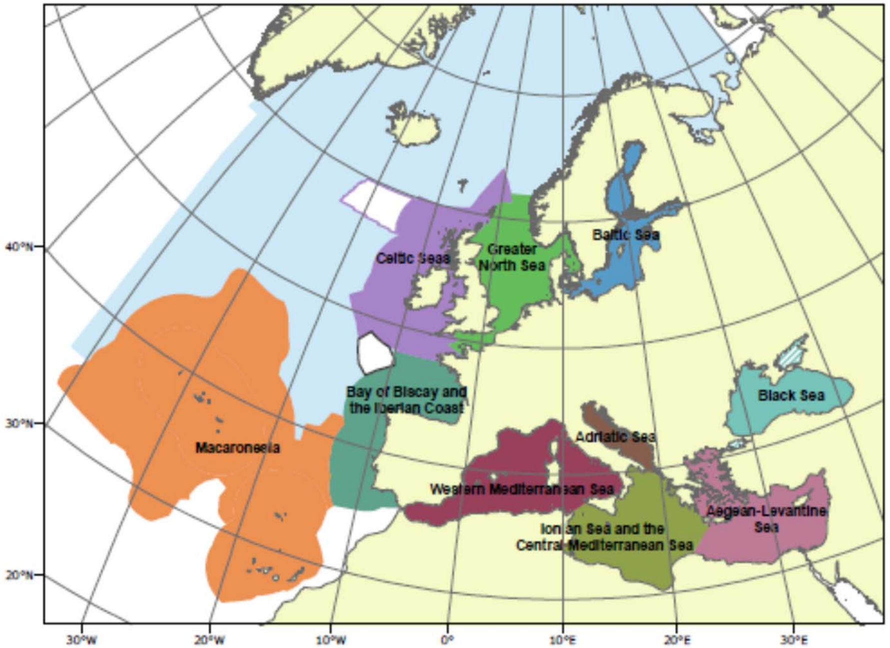
ANNEX V EU Biogeographic Regions and MSFD Subregions

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. Projection of climatic suitability for *Morone americana* establishment

Daniel Chapman
21 May 2018

Aim

To project the climatic suitability for potential establishment of *Morone 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), VertNet, iNaturalist, iDigBio, Ocean Biogeographic Information System (OBIS) and USGS Biodiversity Serving Our Nation (BISON). We removed records where the geo-referencing was too imprecise or estuarine records that were outside the coverage of the terrestrial predictor layers. The remaining records were gridded at a 0.25 × 0.25 degree resolution for modelling (Figure 1a). This resulted in a total of 571 grid cells containing records of *M. americana* for the modelling (Figure 1a), which is adequate for distribution modelling. All records were from North America, and they were divided into native and introduced adventive records using a published native range polygon (NatureServe, 2013).

Climate data were taken from freshwater-specific versions of the 'Bioclim' variables (Domisch et al., 2015) aggregated to a 0.25 × 0.25 degree grid for use in the model. The following variables were used in the modelling:

- <u>Mean upstream temperature of the coldest month</u> (Hydro6°C) reflecting the winter cold stress. Low winter temperatures have been shown to cause very high juvenile mortality (Johnson & Evans, 1991).
- <u>Mean upstream temperature of the warmest quarter</u> (Hydro10°C) reflecting the summer thermal regime. Adults show a behavioural preference for water temperatures between 15 and 30°C (Hall et al., 1979) and larvae do not grow below 13°C (Margulies, 1989; Hanks & Secor, 2011).
- Mean upstream annual precipitation (Hydro12 mm, log+1 transformed) was used as an indicator of the availability of aquatic habitats.

Unfortunately, future scenarios for these variables are not available, precluding assessment of climate change on the potential distribution.

As an additional habitat variable, the <u>proportion cover of inland water</u> (log+1 transformed) was derived from the Global Inland Water database (Feng et al., 2016).

Finally, the recording density of Actinopterygii on GBIF was obtained as a proxy for spatial recording effort bias (Figure 1b).

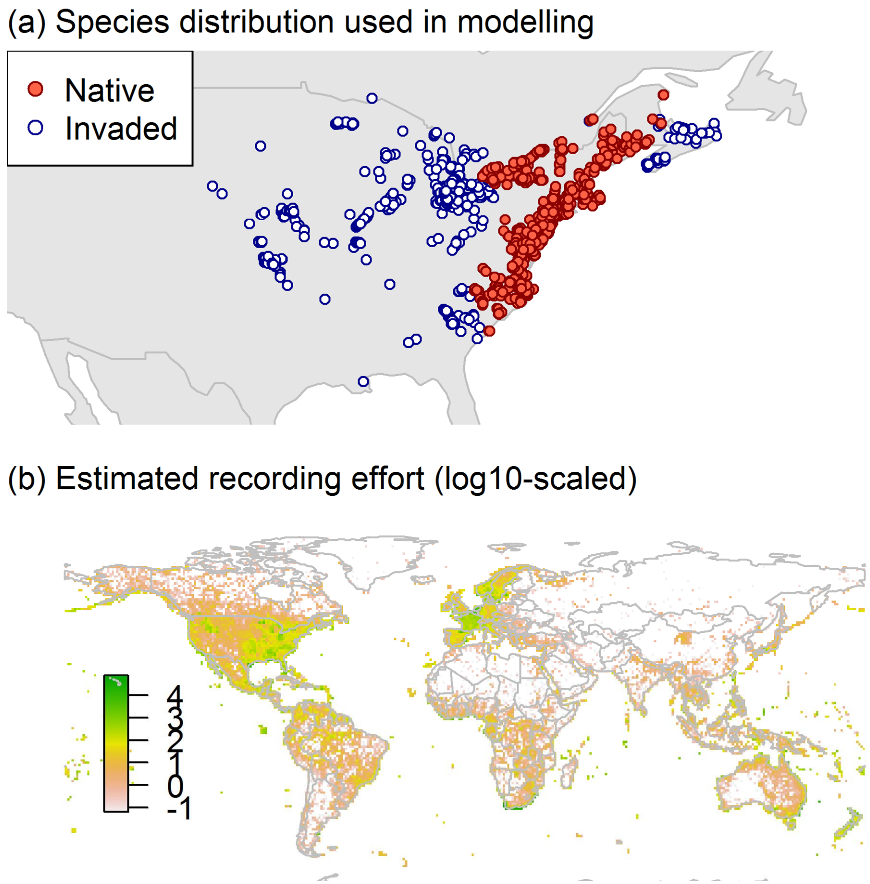


Figure 1. (a) Inland occurrence records obtained for *Morone americana* and used in the modelling, showing the native range and introduced occurrences, and (b) a proxy for recording effort – the number of Actinopterygii records held by the Global Biodiversity Information Facility, displayed on a $\log_{10}$ scale.

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3–7 (Thuiller et al., 2009, 2016). Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale (Elith et al., 2010), we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to. Therefore background samples (pseudo-absences) were sampled from two distinct regions:

- An <u>accessible background</u> includes places close to *M. americana* populations, in which the species is likely to have had sufficient time to disperse and sample the range of environments. The accessible background was defined as both the native range polygon (NatureServe, 2013) and watershed polygons in which the introduced records fell. Watersheds were defined as level 6 polygons from the HydroBASINS dataset (Lehner & Grill, 2013).
- An <u>unsuitable background</u> includes places with an expectation of environmental unsuitability, e.g. places too cold. Absence from these regions should be irrespective of dispersal constraints, allowing inclusion of this background in the modelling. Ecophysiological information suggested that temperature was a key limiting factor, so unsuitable regions were defined based on the extremes of the temperature values at species occurrences:
    - Minimum temperature of the coldest month (Bio6) < –17°C, OR
    - Mean temperature of the warmest quarter (Hydro10) < 14°C, OR
    - Mean temperature of the warmest quarter (Hydro10) > 27°C

Only nine of the 571 occurrences (1.6%) fell within the unsuitable background.
Ten random background samples were obtained:

- From the accessible background 571 samples were drawn, which is the same number as the occurrences. Sampling was performed with similar recording bias as the distribution data using the target group approach (Phillips, 2009). In this, sampling of background grid cells was weighted in proportion to Actinopterygii GBIF recording density (Figure 1b). Taking the same number of background samples as occurrences ensured the background sample had the same level of bias as the data.
- From the unsuitable background 3000 simple random samples were taken. Sampling was not adjusted for recording biases as we are confident of absence from these regions.

Model testing on other datasets has shown that this method is not overly sensitive to the choice of buffer radius for the accessible background or the number of unsuitable background samples.

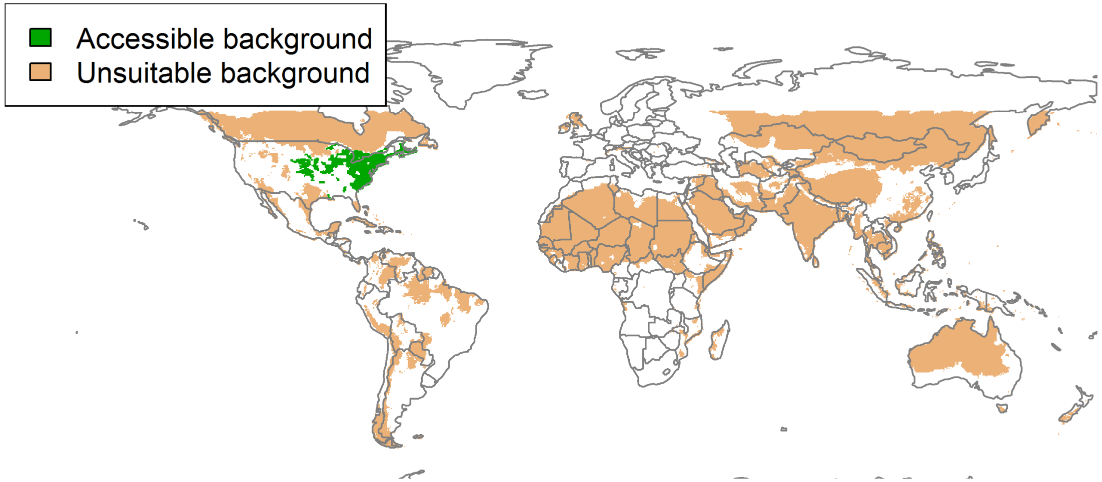


Figure 2. The background regions from which 'pseudo-absences' were sampled for modelling. The accessible background is assumed to represent the range of environments the species has had chance to sample. The unsuitable background is assumed to be environmentally unsuitable for the species.

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 (except where specified below) and rescaled using logistic regression:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per effect.
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent (Phillips et al., 2008)

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 calculating the Area Under the Receiver-Operator Curve (AUC) for model predictions on the evaluation data, which were reserved from model fitting. AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected pseudo-absence.

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.

Global model projections were made for the current climate and for the two climate change scenarios, avoiding model extrapolation beyond the ranges of the input variables. The optimal threshold for partitioning the ensemble predictions into suitable and unsuitable regions was determined using the 'minimum ROC distance' method. This finds the threshold where the Receiver-Operator Curve (ROC) is closest to its top left corner, i.e. the point where the false positive rate (one minus specificity) is zero and true positive rate (sensitivity) is one.

Limiting factor maps were produced following Elith et al. (2010). 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. Partial response plots were also produced by predicting suitability across the range of each predictor, with other variables held at near-optimal values.

Results

The ensemble model suggested that at the global scale and resolution of the model suitability for *M. americana* was most strongly determined by winter and summer temperatures and habitat availability, with little effect of precipitation (Table 1, Figure 3).

Global projection of the ensemble model in current climatic conditions indicates that the native and introduced records from North America fell within regions predicted to have high suitability (Figure 4). The model also predicts that further infilling and westwards range expansion of the introduced North American range is climatically possible, though this will be restricted by the availability of major river systems.

In Europe, most major river systems were predicted as being climatically suitable (Figure 5). The freshwater predictor variables do not extend to the northernmost parts of Europe, but it seems likely that at least southern Scandinavia would be climatically suitable. The model also suggests that suitability for invasion of Europe may be largely limited by the availability of inland water bodies (Figure 6), based on nearly all North American records coming from major river systems. However, if the species is able to colonise more minor rivers in Europe then the species may be able to establish more widely than is shown in Figure 5.

Most European Biogeographical Regions (Bundesamt fur Naturschutz (BfN), 2003) are projected to be suitable for invasion, with the Pannonian and Steppic and Continental regions predicted to be the most at risk in the current climate (Figure 7). However, this analysis may be sensitive to caveats around the distribution of inland water habitat and the northern limit of the predictor variables.

Table 1. Summary of the cross-validation predictive performance (AUC) and variable importances of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the mean values from models fitted to ten different background samples of the data.

Algorithm	**AUC**	**In the ensemble**	**Variable importance**			
			Minimum temperature of coldest month	**Mean temperature of warmest quarter**	**Annual precipitation**	**Proportion inland water**
GLM	0.9458	yes	52%	31%	1%	15%
GAM	0.9454	yes	51%	29%	1%	18%
MARS	0.9429	yes	45%	36%	0%	19%
Maxent	0.9429	yes	38%	32%	3%	27%
GBM	0.9428	yes	29%	47%	0%	25%
ANN	0.9424	yes	56%	22%	4%	17%
RF	0.9247	no	31%	40%	5%	24%
Ensemble	**0.9466**		**45%**	**33%**	**2%**	**20%**

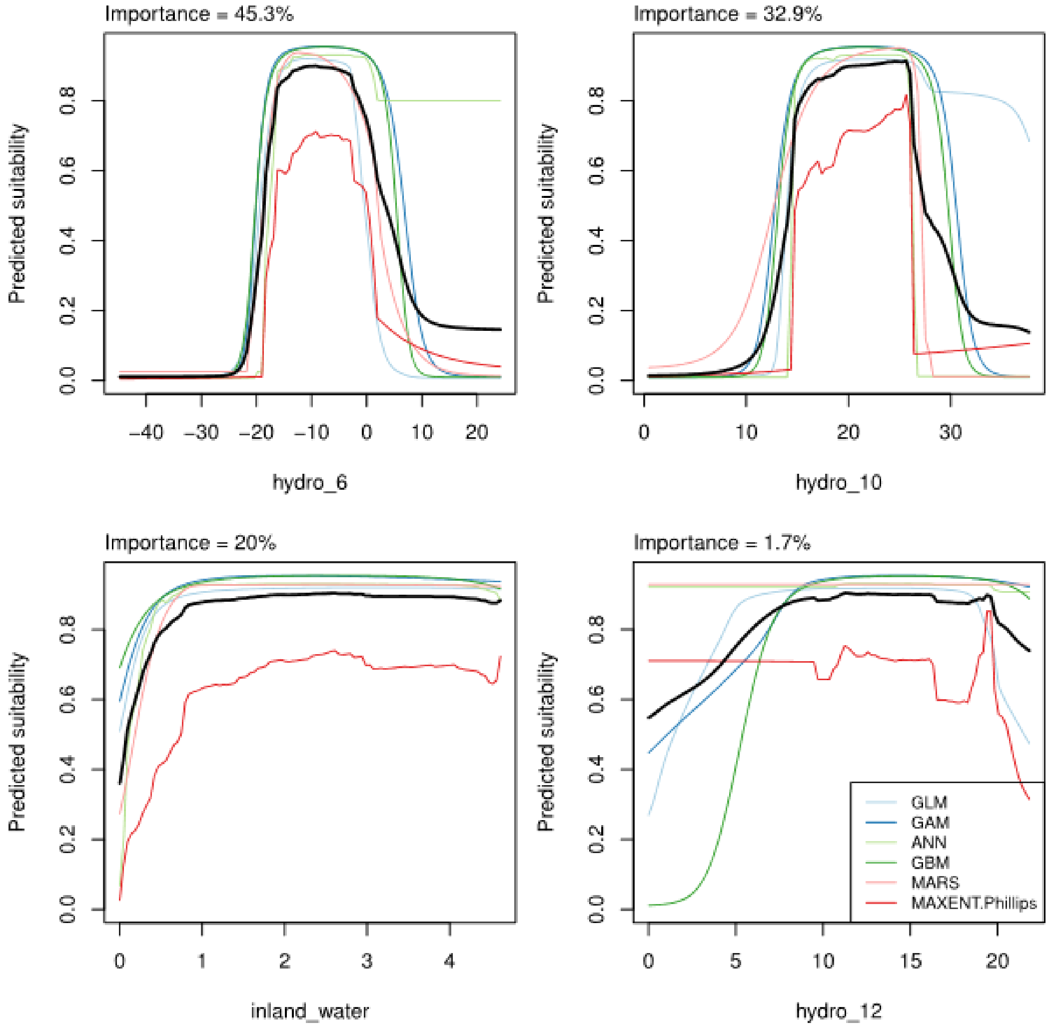


Figure 3. Partial response plots from the fitted models, ordered from most to least important. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

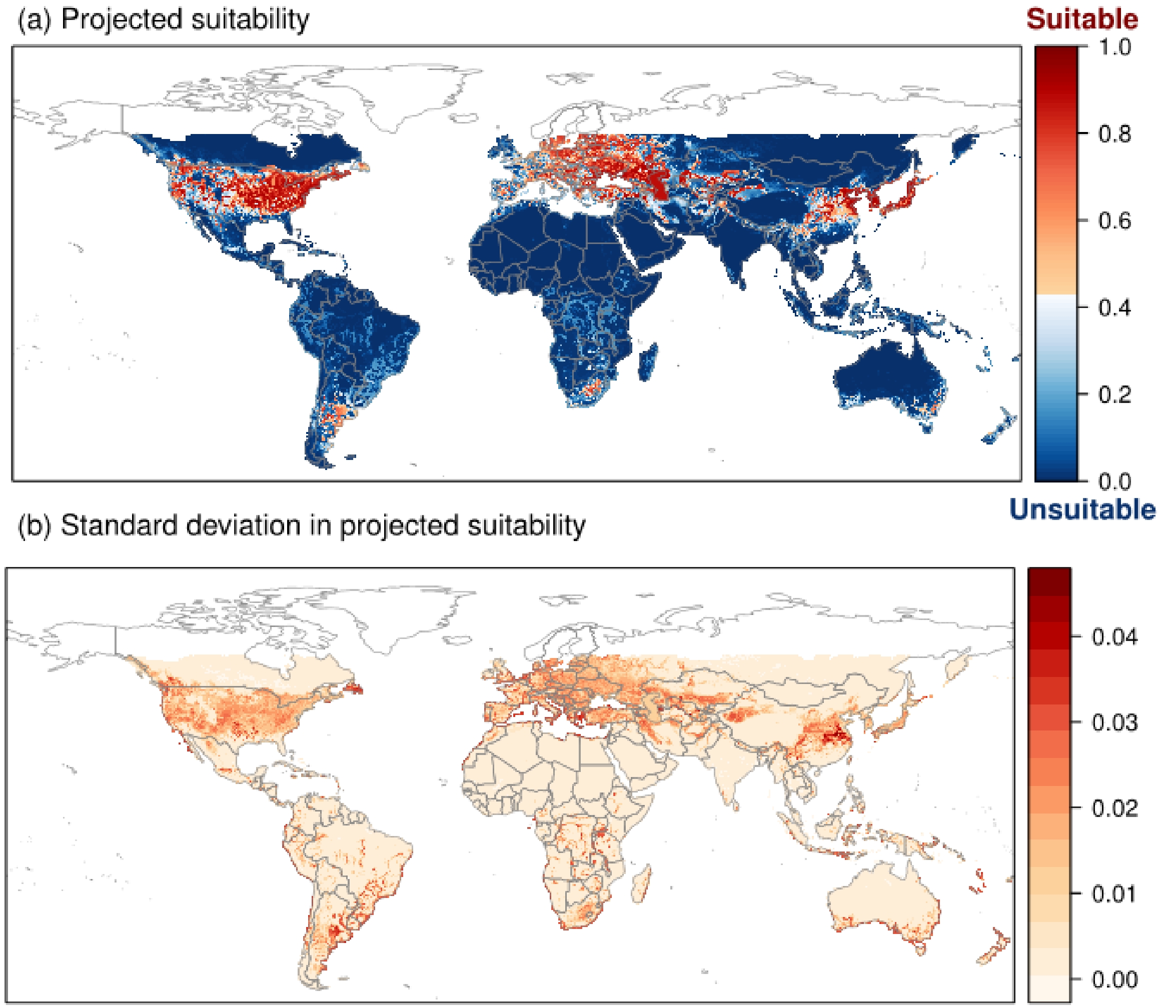


Figure 4. (a) Projected global suitability for *Morone americana* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 × 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Red shading indicates suitability. White areas are beyond the scope of the predictor variables preventing model projection. (b) Uncertainty in the suitability projections, expressed as the standard deviation of projections from different algorithms in the ensemble model.

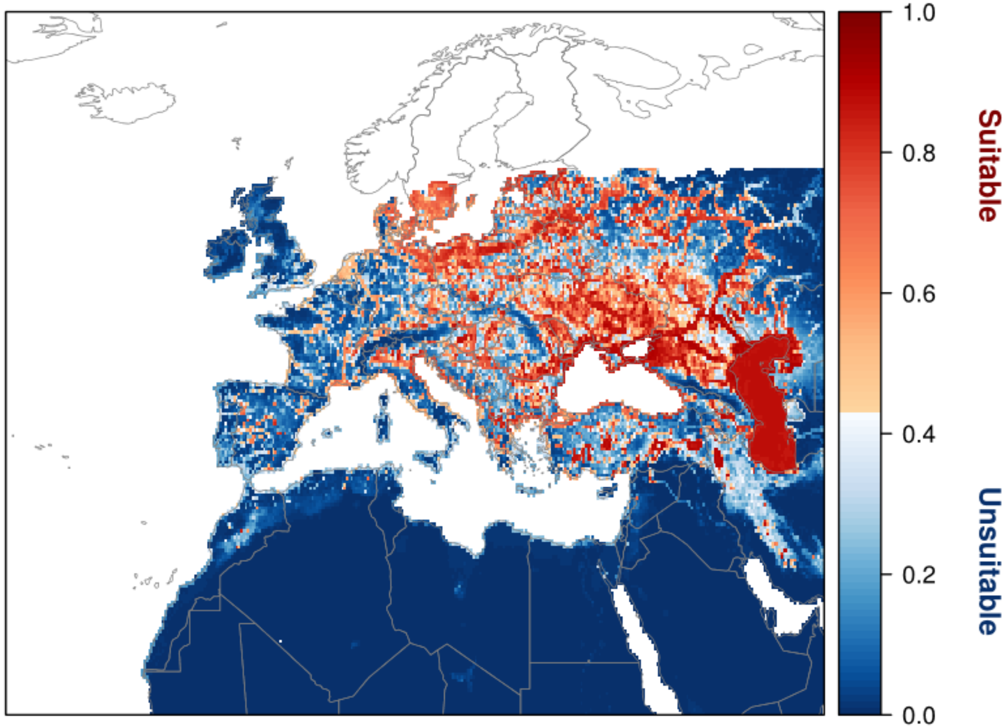


Figure 5. Projected current suitability for *Morone americana* establishment in Europe and the Mediterranean region. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

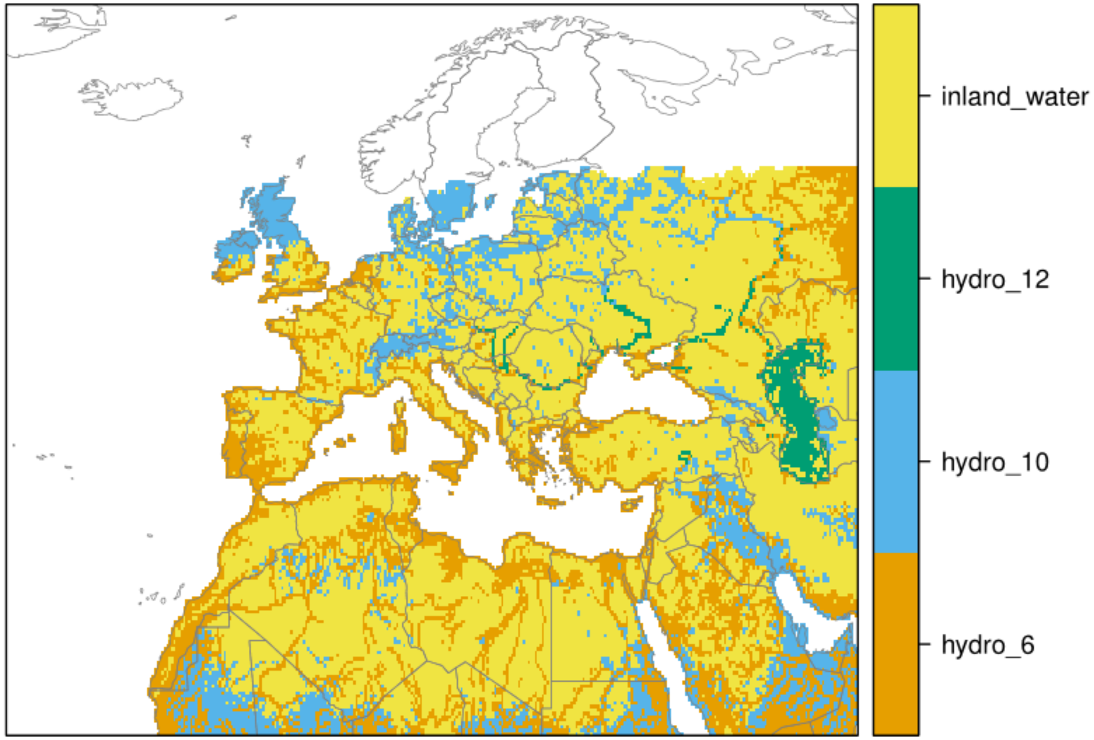
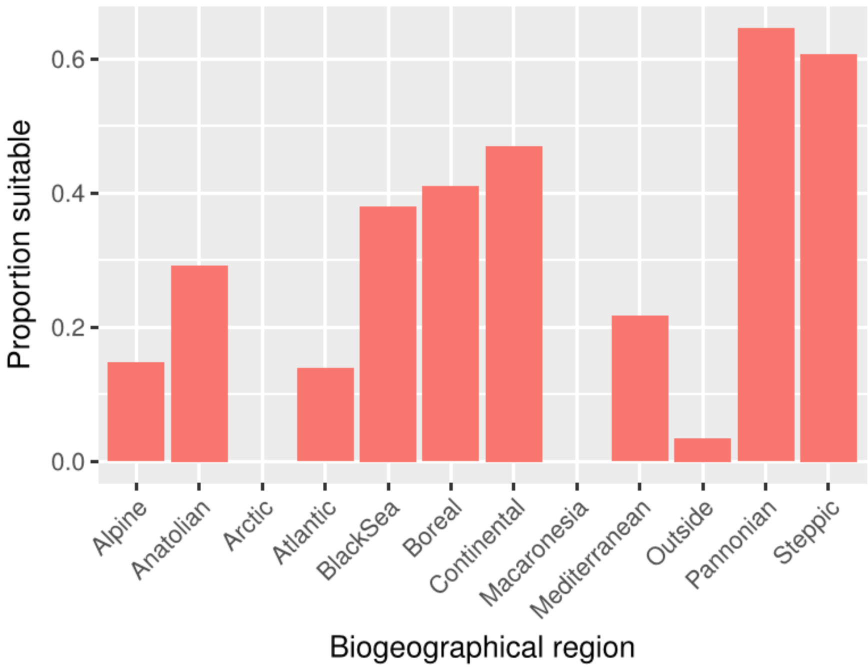


Figure 6. Limiting factor map for *Morone americana* establishment in Europe and the Mediterranean region in the current climate. Shading shows the predictor variable most strongly limiting projected suitability.


Proportion suitable
0.0
0.2
0.4
0.6
Alpine
Anatolian
Arctic
Atlantic
BlackSea
Boreal
Continental
Macaronesia
Mediterranean
Outside
Pannonian
Steppic
Biogeographical region

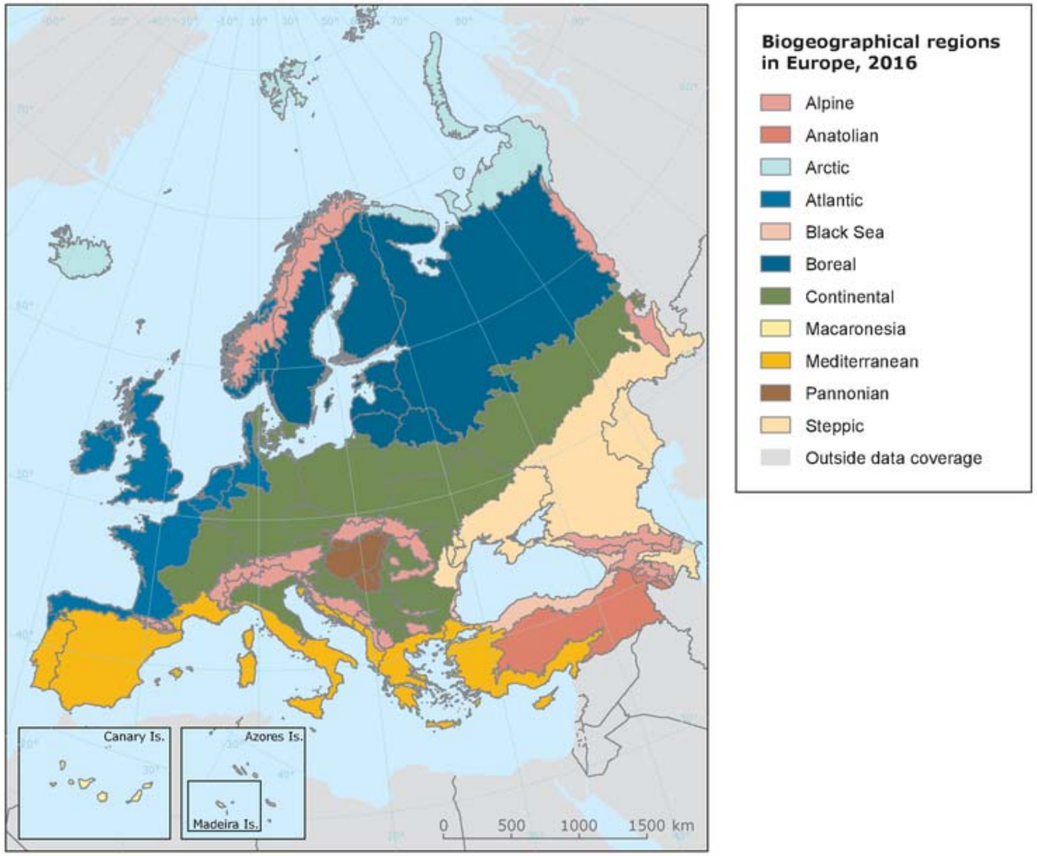


Figure 7. Upper image: Variation in projected suitability among biogeographical regions of Europe. Lower image: map of Biogeographical regions of Europe (map from: www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2)(Use of map copy permitted as per EEA Copyright Notice: www.eea.europa.eu/legal/copyright).

Caveats to the modelling

Modelling the potential distributions of range-expanding species is always difficult and uncertain. The modelling here is subject to uncertainty for the following reasons:

- *Morone americana* exhibits invasive (adventive) behaviour in its native continent, implying that there are strong natural dispersal constraints on the native North American distribution. Even though the modelling tried to account for watershed dispersal constraints, these may have impeded the ability to characterise species-environment responses.
- Despite invasive behaviour in the native continent, there is no record of it invading outside the native continent, including in Europe. *M. americana* is known to be adaptable and capable of acclimation so may be able to expand its niche into cooler or warmer conditions than are currently observed in the native continent.
- The role of inland water habitat as a limiting factor in Europe is especially uncertain.
- The model did not include other variables potentially affecting occurrence of the species, including biotic interactions, salinity or proximity to marine spawning habitats.
- To remove spatial recording biases, the selection of the background sample was weighted by the density of Actinopterygii records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, a number of factors mean this may not be the perfect null model for species recording, especially because additional data sources to GBIF were used.

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Template for Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Morone americana*** **(Gmelin, 1789)**
Species (common name)	**white perch**
Author(s)	**H. Verreycken, L. Aislabie, G.H. Copp**
Date Completed	**24 October 2018**
Reviewer	**Peter Robertson**

Summary

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.

The most cost-effective way of preventing the introduction of white perch *Morone americana* is enforcement of the existing legislation, i.e. the European Council's 2007 Regulation on the use of Aliens in Aquaculture and, if the species is listed on the Annex of the European Council's 2014 Regulation on the Management of Alien Invasive species, then that legislation also. This would effectively reduce the likelihood of entry via the existing vectors and pathways identified in the risk assessment. At the same time, prevention is made more effective when public awareness of the species is raised. By ensuring these measures are carried out, it will help to prevent any introductions to the RA area. Education and awareness campaigns for IAS and their impacts will increase reporting rates and are a cost-effective measure. Working alongside the public and recreational anglers will help to increase the speed of detection, cover a vast area's throughout GB and in the end will be more likely to help to eradicate much more efficiently. The other prevention method for managing pathways is a very expensive challenge and would require certain changes such as ballast water management systems to reduce the risk of introduction.

Early detection of the species within the RA area is very hard due to the wide extend of areas this species inhabits. The only viable way would be reporting of the fish from recreational fisherman and commercial fishing vessels. Although river surveys would be a continuous way of early detection it is a very expensive measure and it is not guaranteed that the fish will travel upstream from estuaries which is their main habitat preference. In the near future, eDNA is likely to provide an means of early detection, however further research is need to render this a viable monitoring/detection method. Regardless, eDNA methods are not able to indicate precisely a species' location, and therefore other methods/approaches would need to be used to locate the species for eradication measures.

Rapid eradication of the species is dependent on the character of the invaded aquatic system. If discovered in a river system, then eradication measures may be applicable. Eradiation methods include the use of a piscicide, rotenone, for which use in the EU is now restricted but still possible for invasive fish species, e.g. *Channa* species. Alternative methods include intensive netting and/or electrofishing, followed by euthanasia of the captured fish. The cost implications of rapid eradication through netting are massive, the amount of labour and time required to carry it out this process increases with the increasing size of the water course or water body, with large rivers and estuaries being aquatic systems in which eradication is highly unlikely to be successful. Indeed, complete eradication of most aquatic species in open water systems, such as rivers or estuaries, is virtually impossible once the species has established itself. So, in terms of estuaries and coastal areas, it may only be possible to create an information programme that asks fishers (recreational and commercial) to kill the species when captured and correctly identified, and then report the capture. There is very limited literature in relation to eradication programmes for *Morone americana* and this has been considered unachievable if established in an open water system. Education of the general public, and especially fishers, is an important contribution to the management of non-native species, assisting in particular with the location of previously-unknown populations in water bodies and water courses. Managing the species would benefit from a better assessment of its impacts. Research on *M. americana* may make it possible to develop other ways of controlling the species, such as the use of a species-specific infectious agent or a less-specific virus that is used in circumstances that will not affect other susceptible species, such as is currently being used to control or eradicate other species, e.g. common carp *Cyprinus carpio* in Australia, however, such an approach would require considerable investment. Natural native predators could possibly reduce numbers dependent on size of the fish as species, such as northern pike *Esox lucius* and pikeperch species (*Sander* species) are known predators of *M. americana.* However, bio-resistance in the form of predation is rarely (if ever) successful in open waters, and has long been known to play only a limited role in resisting a fish species' establishment. *Morone americana* is known to respond (i.e. avoid) strobe-light deterrents, so it may be possible to reduce the species distribution using this deterrent. Without a significant amount of investment, it is very unlikely that there will be a successful method of managing this species.

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	**Managing the pathways:** The white perch is not yet present in the RA area. We have identified	Estimates of costs for management of these pathways are likely to be very high (e.g. >10 million EUR) for the RA area.	High

	three possible pathways of introduction relevant to Europe: i) as transport stowaway with ballast water, ii) as a transport contaminant on animals i.e. for aquaculture and iii) for stocking for recreational angling. The adoption and enforcement of appropriate legislation and codes of best practice could reduce the likelihood of introduction. For pathway i) the new regulation for ballast water treatment is in force since 2017 (Ballast Water Convention) so the potential of introduction via ballast water would be further limited. Pathway ii) could be better controlled by extensive checking of live fish transports and a ban of live sale would be an effective means of limiting the risk of introduction of the species through the pathway iii.	In particular, installation costs of ballast water management systems can be considerable – estimates by ship-owner organisation BIMCO suggest up to $5 million (USD) per ship – and operational costs of the systems over the ship's lifetime could be even higher (www.ballastwatermanagement.co.uk). Treatment of ballast water should be very effective in reducing the risk of introducing *M. americana* in the RA area, and avoidance of the species being taken up where ballast water is being taken on could be reduced by the use of strobe-light deterrents (Sager et al., 2000). Also better control and extensive checking of live fish transports (to detect stowaways) and a ban of live sale of *M. americana* would be an effective means of limiting the risk of introduction of the species through the pathways ii) and iii) Estimates of yearly costs in the RA area associated with these last two measures are likely to be moderately high (€200k–1M) and concern mainly extra man hours for intensive control and for training and educating staff to recognize unwanted specimens.	
	Increasing public awareness: the species is not yet being imported in the RA area for aquaculture or sport fishing. Other species and hybrids of the genus *Morone*,	Campaigns to educate and increase awareness on IAS are an effective way to curb illegal introductions and increase reporting rates, especially those targeted at specific sectors (García-Llorente et al., 2011). Public awareness campaigns, however, do need to be maintained so they do not drop out	High

	however, are in aquaculture in several European countries.	of the collective consciousness, but also renewed periodically to avoid fatigue. Estimates of costs of campaigns to increase awareness are likely to be low to medium (e.g. €50–200K) on a European scale. Campaigns created through social media are low cost, and there is no cost associated with a news network. However, costs would increase if advertisements were placed in newspapers and/or magazines.	
Methods to achieve eradication	**Effective surveillance and reporting:** *Morone americana* is a readily identifiable species although it may be confused with *M. chrysops* (white bass). The *Morone* genus also forms hybrids (e.g. *M. americana* x *M. chrysops* and also *M. chrysops* x *M. saxatilis*, of which the latter has been imported to some EU and neighbouring countries for aquaculture) which may be difficult to identify. 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. Post-eradication detection can also be undertaken to determine	Trawl nets, fyke nets, traps and electrofishing can be used for surveillance and monitoring in the RA area, even if not always effective at low density. Also, eDNA has been used to successfully detect non-native species at low density (Dougherty et al., 2016), even in large lakes (Larson et al., 2017). Citizen science could be promoted to monitor the possible introduction and spread of the species. Estimates of costs of dedicated surveillance and monitoring and subsequent removal of this non-native fish species from the RA area are likely to be moderately high (e.g. €200K–1M).	High

	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, border control staff, aquarists) to increase the probability of an early detection and rapid response		
	Use of piscicide: 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.	*M. americana* can easily be killed by rotenone (acute toxicity to *M. americana* was anticipated to be within recommended concentration levels on product label for similar fish and was corroborated by laboratory bioassay (LC100 of 0.15 mg/L; Wujtewicz et al., 1997) or other piscicides. *M. americana* typically inhabit coastal, brackish and transitional waters such as estuaries. The use of rotenone in such large open environments is neither practical or desirable and eradication would be unlikely to succeed (Williams & Grosholz, 2008). Indeed, water body size is one of the criteria used in the UK to determine the feasibility and likelihood of success in the use of rotenone to eradicate non-native fishes (Britton et al., 2011). If *M. americana* were to be introduced in to enclosed water bodies such as ponds or lakes, then eradication may be feasible. However, the use of rotenone has significant effects on other species and issues of public acceptability which would need to be carefully considered.	Medium

		Estimates of costs of the application of rotenone or another piscicide could be low-to-medium (e.g. €50–200K), depending on what area has to be treated (Britton et al., 2011). If eradication is deemed potentially effective, then its effectiveness may be enhanced by using deterrents, e.g. strobe lights (Sager et al., 2000) to keep *M. americana* restricted in the location where the eradication measures are to be implemented.	
Methods to achieve management	**Raising awareness:** Raising public awareness of the risks posed by invasive alien species in general and *M. americana* in particular. Should the species become established in an area, targeted information to raise awareness could be used to help reduce the risk of local spread or transportation, for example by targeting commercial and recreational fishers.	Sea Grant Programmes in the USA have been raising awareness and brining invasive species outreach for several decades and seem to be very successful. The costs for outreach and production of leaflets can be moderately high (€200–1M) when applied across a large community, such as in Europe. However, if this outreach could be focused on the likely area of establishment, then estimates of costs would be low-to-moderate (e.g. €50–200K).	High
	The **above methods** described to support eradication can also be used to manage existing *M. americana* populations.	See above	See above
	Reducing risks of further dispersal should the species become established e.g. reducing abundance by introduction of predators,	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 white perch and to ensure these water courses are not recolonised by the species after eradication.	Medium

	targeted fishing, netting and fyking or the introduction of pathogens.	The further dispersal may be reduced by the presence of existing predatory fishes, such as northern pike *Esox lucius* and/or pikeperch *Sander lucioperca*. The introduction of these species as biocontrols would, however, only be adequate in closed waters in parts of the EU where they are native. In riverine environments targeted fishing efforts (angling, fyke or seine netting) could be employed to keep the white perch restricted to a certain area. This would imply high labour costs over several years. The introduction of pathogens is also a way to reduce the abundance of white perch but this method is still very controversial in many parts of the world. Depending on the area that has to be monitored, estimates of the management costs may be moderate-to-very high (e.g. from <€5k to > €1M).	

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2[1]
Name of organism: ***Perna viridis*** **Author(s) of the assessment:** Jack Sewell, The Marine Biological Association, UK Paul Stebbing, Cefas, UK Phil Davison, Cefas, UK **Risk Assessment Area:** The risk assessment area is the territory of the European Union, excluding the outermost regions. **Peer reviewer 1:** Marika Galanidi, Dr, University, Institute of Marine Sciences and Technology, Izmir, Turkey **Peer reviewer 2:** Argyro Zenetos, Dr, Hellenic Centre for Marine Research, Greece This risk assessment has been peer-reviewed by two independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study. **Date of completion:** 22/09/18

[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

Contents

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**[2]	**COMMENT**
Summarise Entry[3]	Likely	High	The most likely pathways of entry are considered to be via ballast water or via hull fouling. Many of the unintentional introductions globally have been attributed to ballast water. For example, *P. viridis* in Tampa Bay Florida is believed to have been introduced as larvae via ballast water transfer and a population is now well established in the area (Rajagopal et al., 2006). There is evidence to suggest that the successful Introduction of *P. viridis* to Jamaican waters was via ship ballast water introduction of larvae (Buddo et al., 2003).The life history and wide environmental tolerances) of the species make introduction via this pathway. *P. viridis* is capable of attaching to multiple surface types using byssal threads and is capable of remaining attached in very strong currents (Rajagopal *et al.*, 2006). It is therefore highly likely to attach to vessel hulls in its current range. Many cargo and recreational vessels move from the known range to the RAA daily and introduction with these vessels either in ballast or attached to hulls is considered moderately likely. *P. viridis* is commercially valuable as a food species and has been intentionally introduced into Southwest China, New Caledonia, Fiji, Tonga, Tahiti, Western Samoa, Japan, Cook Islands and Cape Verde Islands. All but two (Cape Verde and Cook Islands) have resulted in successfully established populations (Baker et al., 2007). Legislation in the RAA should prevent legal introductions, and the authors therefore consider introduction via this pathway to be unlikely at present.

[2] In a scale of low / medium / high, see Annex III

[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

			However illegal introductions and introductions to neighbouring states with different legislation may result in established populations. Additionally, any changes to the relevant legislation in the future may change the score given.
Summarise Establishment[4]	Likely	High	*P. viridis* is capable of colonising a range of substrates and is highly tolerant of varying temperature, salinity and turbidity. Mortality caused by lowest winter sea temperature is likely to be the most important factor controlling the northward spread of *P. viridis* in the RAA. Cold winter air temperatures (<2°C) are also likely to cause mortalities in intertidal populations but would be less likely to impact subtidal populations. Salinity levels are suitable throughout, with the exception of the Baltic and Eastern Black Sea, where Salinity is below the 15ppt lowest tolerable level. The likely minimum sea temperature thresholds are thought to be 10°C and 13°C for survival and reproduction respectively. Sea temperatures exceeding the maximum survivable temperature (35°C) do not occur in the RAA. Parts of the Eastern Mediterranean fall within the 26 – 32°C optimal sea temperature range in July and August, meaning that reproductive rates in these regions could be high even in current conditions. And establishment could be more likely. Suitable habitat is widespread in the RAA and competition and predation is considered unlikely to impair establishment, especially in areas where conditions are optimal growth and reproduction. *P. viridis* is a highly fecund, fast growing species, tolerant of a wide range of environmental conditions and is able to capitalise on environmental change and events which may displace other species. It is also capable of attaching to and overgrowing existing

[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

			organisms. It is capable of year-round reproduction, although this does not occur in all populations and females are capable of releasing 3 to 6 X 10^6 (3-6 million) Or rarely 19 × 10^6 (19 million) eggs in a single spawning event, potentially leading to rapid establishment if introduced. Evidence from interceptions in Australia where the species has been unable to establish despite numerous introductions suggests other, as-yet-unknown biological factors which may impair establishment and might be relevant to the RAA.
Summarise Spread[5]	Rapidly	Medium	It is considered that due to the high fecundity and long-lasting, pelagic larval phase, natural larval dispersal and spread once introduced and established is likely to be rapid. Anthropogenic spread via fouling and ship ballast is also considered likely and the large amounts of passenger and cargo vessels moving regularly and freely within the RAA is likely to facilitate this process. Similarities with native species of mussel increase the likelihood that adult mussels will remain undetected further facilitating spread.
Summarise Impact[6]	Major	Medium	Predicted environmental impacts are based on studies outside the RAA. It is considered likely that *P. viridis* has the potential to displace native species, particularly other mussel species. The dense turf formed by the species has the potential to smother native species and alter existing habitats. However data to support these predictions is extremely limited. There is evidence to suggest that predation by *P. viridis* on zooplankton and phytoplankton might impact pelagic trophic systems, which may include a number of ecologically, commercially and socially important

[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly

[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

			species. Habitats of conservation importance, including seagrass beds, reefs and mudflats are most likely to be impacted. The ecosystem services associated with seagrass beds and biogenic reefs are likely to be impacted should these species be displaced, smothered or otherwise impacted by *P. viridis*. These services include coastal defence, provision of food, sediment stabilisation and provision of habitat for commercially and culturally important species. *P. viridis* is known to be a serious fouling pest species. In particular, costs associated with removal from cooling pipes and reduced efficiency caused by fouling in power stations are likely to be high. There is some evidence to suggest that *P. viridis* is able to smother commercially important species, including oysters and other species of mussel. These fisheries are of significant commercial importance in the RAA and any impact would have serious economic and social consequences.
Conclusion of the risk assessment[7]	High	Medium	*Perna viridis* exhibits a number of traits, which have led to successful introduction, establishment and spread globally. Although not yet recorded in the RAA, these traits, combined with the presence of potential pathways of entry, make introduction likely. Once introduced, tolerable conditions throughout the Mediterranean and on the Atlantic coast of Spain and Portugal, combined with the adaptable, tolerant nature and high fecundity of *P. viridis* increase the likelihood of successful establishment and spread. Current conditions in the Mediterranean and Atlantic coast of Spain and Portugal are currently within the tolerable range of temperature and salinity. It is likely that the habitable area will increase as seas become warmer and that the tolerable areas will become even more optimal. Once established,

[7] In a scale of low / moderate / high

			environmental, economic and social impacts are likely to be high, with particular impacts on native sessile organisms and a range of habitats. As a prolific fouling organism, impacts on shipping, fishing and industrial cooling structures are likely to result in high economic costs.

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Belgium	-	-	-	-
Bulgaria	-	-	?	-
Croatia	-	-	yes	-
Cyprus	-	-	yes	-
Denmark	-	-	-	-
Estonia	-	-	-	-
Finland	-	-	-	-
France	-	-	yes	-
Germany	-	-	-	-
Greece	-	-	yes	-
Ireland	-	-	-	-
Italy	-	-	yes	-
Latvia	-	-	-	-
Lithuania	-	-	-	-
Malta	-	-	Yes	-
Netherlands	-	-	-	-
Poland	-	-	-	-

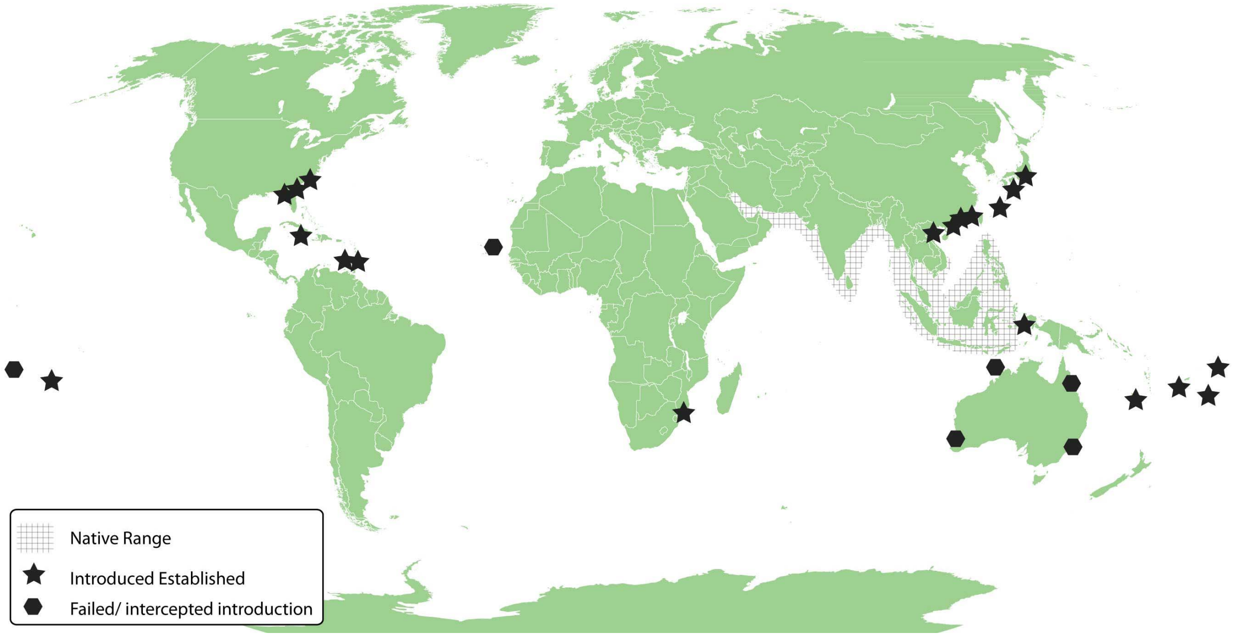
Portugal	-	-	Yes	-
Romania	-	-	-	-
Slovenia	-	-	yes	-
Spain	-	-	yes	-
Sweden	-	-	-	-
United Kingdom	-	-	-	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Baltic Sea	-	-	-	-
Black Sea	-	-	?	-
North-east Atlantic Ocean	-	-	Yes	-
Bay of Biscay and the Iberian Coast	-	-	Yes	-
Celtic Sea	-	-	?	-
Greater North Sea	-	-	?	-
Mediterranean Sea	-	-	Yes	-
Adriatic Sea	-	-	Yes	-
Aegean-Levantine Sea	-	-	Yes	-
Ionian Sea and the Central Mediterranean Sea	-	-	Yes	-
Western Mediterranean Sea	-	-	Yes	-

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Species: *Perna viridis* (Linnaeus, 1758) Class: Bivalvia Order: Mytilida Family: Mytilidae Synonyms (World Online Register of Marine Species April 2018) *Mytilus viridis* Linnaeus, 1758 *Mytilus smaragdinus* Chemnitz, 1785 *Mytilus smaragdinus* Gmelin, 1791 *Mytilus opalus* Lamarck, 1819 *Chloromya smaragdinus* Jukes-Browne, 1905 *Chloromya viridis* Dodge, 1952 Names used in commerce (En) Green-lipped mussel; (En) Asian green mussel; (En) green mussel (fr) moule verte asiatique; (NL) Aziatische groene mossel; (De) Asiatische grüne Miesmuschel a list of the most common subspecies, lower taxa, varieties, breeds or hybrids Non known
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 wild, 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. Mytilid mussels, including commercially and environmentally important species such as *Mytilus edulis* and *Mytilus galloprovincialis*. Horse mussel *Modiolus modiolus*. There are three known extant species in the genus Perna all are similar in appearance to *P. viridis*. (Wood et al., 2007). The Atlantic congener *Perna perna* (Brown mussel) has been recorded from Portuguese waters and has limited distribution in the South West Mediterranean it is also present in the Red Sea. This species can be very similar in morphology and colour to *P. viridis* but can be separated by examination of mantle pappilae and palial line (Micklem et al., 2016). *P. picta* is considered a Mediterranean ectomorph of *P.perna* and not - as previously suggested - a separate species (Wood et al.,

	2007, Callapez et al., 2012) and is also morphologically similar to *P. viridis*. The third species in the genus *P.canaliculus* is native to New Zealand and is exported and sold globally alive and frozen as a valuable food commodity.
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	Both: CABI Datasheet – 'Global Review of impacts, spread and biology of *P. viridis*'; and the ISSG (Invasive Species Specialist Group (ISSG) of the IUCN Species Survival Commission) Global Invasive Species Database (GISD 2015) provide broad global relevance including RAA but does not specifically consider features of RAA. Heersink et al. (2014) have undertaken an assessment of the of *P. viridis* becoming invasive in Australia.
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 (From CABI 2018) "The native range of *P. viridis* is along the Indian coast and throughout the Indo-Pacific (Siddall, 1980). It is broadly distributed in the Indo-Pacific where it ranges west from the Persian Gulf and east to New Guinea and Japan and New Guinea for north and south ranges, respectively. *P. viridis* occurs naturally and is widely distributed along the intertidal coasts of India (Jones and Alagarswami, 1973). It is also local to Malaysia (Sivalingam, 1977) and rock stacks on the Mangalore coast of India (Kuriakose and Nair, 1976). Figure 1: Native and introduced range of *Perna viridis* (Based on Baker et al, 2007 + additional records)

	 Based on the native range described and length of planktonic larval phase, it is very unlikely that natural spread into the RAA will occur.
A5. What is the global non-native distribution of the organism outside the risk assessment area?	*P. viridis* has been introduced to and is now distributed in the Central Atlantic and Northwest Pacific. Specifically the following countries: Hong Kong; Japan; USA (Florida, Georgia, south Carolina, Washington); Jamaica; Trinidad and Tobago; Venezuela; Australia ; South Africa; and Fiji (CABI 2018, Micklem et al., 2016). It has also been intentionally introduced to Southwest China, New Caledonia, Fiji, Tonga, Tahiti, Western Samoa, Japan, Cook Islands and Cape Verde Islands.
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?	*P. viridis* has not yet been recorded in the RAA, and therefore it is assumed not to have become established.
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?	**Current climate:** ***Marine regions:*** North-east Atlantic Ocean, Mediterranean Sea, Black Sea (far west) ***Marine subregions:***

	Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea & Central Mediterranean Sea, Aegean-Levantine Sea, Black Sea. **Future climate:** (50 – 100 years, based on RCP 4.5 and RCP 8.5) ***Marine regions:*** North-east Atlantic Ocean, Mediterranean Sea, Black Sea ***Marine subregions:*** English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea, and Black Sea (Sea of Marmara). **Explanation** From the Bay of Biscay, northwards, winter SST currently drops below the Apparent survival threshold of 10°C and reproductive threshold of 13°C suggested by Gilg et al. (2014) and therefore, under current conditions areas north of La Rochelle, France would currently be unlikely to support establishment of *P. viridis*. However, based on potential climate change scenarios (RCP 4.5 and RCP 8.5: 0.6 – 2.5°C in 50 years and 1.4 – 5.8°C in 100 years), winter temperatures may become tolerable in the entire Bay of Biscay and Brittany coast (Celtic Sea and English Channel) within the next 50 -100 years. Maximum summer temperatures do not exceed 32°C, which is the maximum temperature found in currently inhabited range (Power et al. 2004, Urian et al., 2011) in any part of the RAA (Emodnet data – see appendix). Suggesting high-seawater-temperature-related mortality is unlikely to be an issue which impairs establishment. Mean summer temperatures do not currently fall within the optimal range of 26 – 32°C described by Power et al. (2004) in most parts of the RAA. Suggesting that – with the exception of some parts of the southern Mediterranean - establishment may be slower and less likely than in tropical regions. This temperature range represents many areas where probability of establishment may be reduced, such as the north part of the Bay of Biscay, northern Adriatic, the Gulf of Lyon and even North Aegean. An exception is in the Eastern Mediterranean, on the Levantine coast (outside the RA area) where temperatures reach and exceed 26°C (average monthly T) in June, while parts of the RAA in the Eastern Mediterranean fall within the 26 – 32°C threshold in July and August, meaning that reproductive rates in these regions could be high even in current conditions. Based on future warming scenarios (RCP 4.5 and RCP 8.5: 0.6 – 2.5°C in 50 years and 1.4 – 5.8°C in 100 years), if maximum predicted temperatures are realised, all parts of the Mediterranean, and the

	Atlantic Coast of Europe from Spain to the northern Bay of Biscay could fall into this optimum temperature range within the next 50 to 100 years. Current annual temperature ranges throughout the Mediterranean and the coasts of Portugal and Atlantic Coast of Spain and to the northern Bay of Biscay fall within the range of temperatures *P. viridis* experiences in its native range, which are between 12 and 32ºC (Urian et al., 2011). Salinity levels comparable to native range and within known tolerance are also found within the RAA (see map in appendix: Emodnet data). *P. viridis* is euryhaline and can comfortably tolerate salinities ranging from 15ppt – 35ppt (Nicholson 2002). *P. viridis* does not appear to be capable of tolerating salinities lower than 15ppt (McFarland 2013). Studies have show that *P. viridis* is able to tolerate salinities of up to 80ppt with 50% mortality with an optimum salinity for growth between 27 and 65ppt (Sivalingam 1977). These levels suggest that *P. viridis* would be able to tolerate and even thrive at higher salinity levels (>39ppt) found in parts of the Mediterranean. In the Eastern Black Sea, current salinity levels and winter temperatures are lower than those required for the survival of *P. viridis.* The western region of the Black Sea is currently within habitable ranges. Future predicted warming and increased salinity associated with reduced freshwater input may extend the potential habitable area of the Black Sea. Salinity and temperature in the Baltic are not currently suitable to sustain populations and if salinity decreases in this area as models predict (see appendix), the area will continue to be unable to support *P. viridis* populations in the future. The entire Mediterranean currently remains at a temperature tolerable to *P. viridis* throughout even coldest recorded winters. The Black Sea and Atlantic Coast above the southern half of the Bay of Biscay currently drop below the 10ºC threshold during winter, and 13oC in early spring – this being the spawning threshold estimated based on findings of Gilg et al. (2014). These parameters suggest the areas would not be suitable for the species' survival in current conditions. However, Given the future predicted warming scenarios (0.6 – 2.5ºC in 50 years and 1.4 – 5.8ºC in 100 years), the area habitable to *P. viridis* may move northwards in the next 50-100 years to include the Atlantic Coast through the Bay of Biscay and up to the Celtic Sea (French coast) and south-west English Channel (French Brittany Coast). *P. viridis* is capable of colonising a range of substrates and is highly tolerant of varying temperature, salinity and turbidity. Mortality caused by lowest winter sea temperature is likely to be the most important factor controlling the northward spread of *P. viridis* in the RAA. Increasing winter temperature is likely to lead to improved winter survival where temperatures do not drop below 10ºC to

	14ºC. Increased summer temperatures will also increase the habitable and optimal range of the species and is likely to enhance reproductive output, even in currently habitable areas. For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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.	*P. viridis* has not yet been recorded in the risk assessment area and is therefore not established in any EU member state.
A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	**Current climate:** Bulgaria, Croatia, Cyprus, France, Greece, Italy, Malta, Portugal, Slovenia, Spain **Future climate:** (50 – 100 years, based on RCP 4.5 and RCP 8.5) Bulgaria, Croatia, Cyprus, France, Greece, Italy, Malta, Portugal, Romania, Slovenia, Spain (See A7 for explanation)
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?	Yes. Impacts outside of the native range and outside of the RAA are reviewed by Rajagopal et al. (2006), CABI (2018) and include, fouling of industrial cooling pipes, vessels and other man-made structures. Competition with and displacement of native species, including *Perna perna/ picta*, which is naturally present in parts of the South West Mediterranean. Rajopal et al. (2006) provide evidence to support the theory that *P. viridis* (especially in dense aggregations) has the potential to alter plankton dynamics in coastal systems. Another potential impact discussed by Rajagopal et al. (2006) is the release of nitrogenous and phosphorous wastes from dense populations, which can be very high and have the potential to alter nutrient dynamics within water bodies, promoting algal blooms and bays and more enclosed areas.
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	*P. viridis* has not yet been recorded in the RAA
A12. In which EU member states has the species shown signs of invasiveness?	*P. viridis* has not yet been recorded in the RAA
A13. Describe any known socio-economic benefits of the organism.	**Food** Due to its fast growth rate and large size, *P. viridis* is a commercially valuable and important species throughout its native range. With a global aquaculture production of 159,474 tonnes in 2014 and a smaller wild capture fishery (approx. 4,000 tonnes in 2014)(FAO 2018). The species is a prized food in

	China, Philippines and Malaysia (FAO 2018). Countries within the RAA most likely to benefit from the opportunity provided are those with coastal areas currently enjoying optimal growth conditions (summer temperatures between 25 and 32ºC) currently those bordering the Tyrrhenian, Ionian & Levantine basins. There are some concerns over food safety due to the ability of the species to accumulate toxins, including heavy metals from the surrounding water (Rajagopal et al., 2006). But these are concerns likely to apply to many other species of commercially important marine bivalve species in Europe and fisheries would be subject to the same rigorous checks and water quality regulations as these existing fisheries. Commercial *P. viridis* farming in India has had significant socioeconomic benefits, raising employment and wealth, increasing technological development and empowering women (Kripa & Mohamed 2008). **Bioindicator** *P. viridis* is considered to be one of the best mussel species to test for bio pollution. It therefore has potential value as a bioindicator. This is in part due to its relatively high tolerance to heavy metal organochlorine and petroleum hydrocarbon contaminants (Putri et al., 2012, Tanabe et al., 1987, Philips 1985).

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."
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	Few (3)	Medium	Introduction within ship's ballast is considered the most likely cause of the Caribbean/ USA invasion, although examples of documented evidence of ballast contamination could not be found. The most commonly evidenced pathway other than intentional introduction

[8] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf
[9] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

			for farming/human consumption has been as vessel fouling. Due to the potential commodity value of the species, it has been intentionally introduced in the past to areas outside the RAA and this is therefore considered as a potential pathway for movement of the species into the RAA. The US National Parks Authority (2018) suggests Animal Trade (currently sold in the aquarium trade in the USA and used for bait by anglers) and introduction for research as bioindicator may be additional potential pathways. However, these have not been considered here as we do not believe initial introductions to the area by these pathways would occur. However, human induced secondary spread via these pathways is possible. Introduction of live individuals into captivity for food or on growing and subsequent escape was considered, but no evidence could be found to suggest that the species is currently being imported or exported globally in this way, particularly into the RAA nor was there evidence of plans to do so. This pathway has not therefore been reviewed as an active pathway. If escape from confinement was to be considered, this would, in most cases need to be human mediated (intentional release) unless introduction was to an open system leading to escape of larvae – this has been considered alongside RELEASE IN NATURE: Fishery in the wild.
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each	1. TRANSPORT - STOWAWAY: Ship/ Boat Ballast water 2. TRANSPORT - STOWAWAY: Ship/ Boat Hull fouling		

question if you consider more than one pathway, e.g. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	3. RELEASE IN NATURE: Fishery in the wild		
Pathway name:	**TRANSPORT - STOWAWAY: Ship/ Boat Ballast water**		
1.3. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	Unintentional	Medium	Many of the unintentional introductions globally have been attributed to ballast water. For example, *P. viridis* in Tampa Bay Florida is believed to have been introduced as larvae via ballast water transfer and a population is now well established in the area (Rajagopal et al., 2006). It has been speculated that the successful Introduction of *P. viridis* to Jamaican waters was via ship ballast water introduction of larvae (Buddo et al., 2003).
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	likely	Medium	Mussels are likely to inhabit man-made structures within native and non-native ranges, including harbours and docks. Due to food availability and other favourable conditions, these aggregations are likely to occur close to the surface and within range of vessel hulls and ballast inlets (Baker et al. 2007). *P. viridis* reach reproductive age rapidly (2-3 months) and are highly fecund broadcast spawners, with females capable of releasing 3 to 6 X 106 (3-6 million) Or rarely 19 × 106 (19 million) eggs in a single spawning event (McFarland et al., 2016) capable of gametogenesis throughout the year in suitable conditions (McFarland et al., 2016, Rajagopal et al., 2006; Kripa et al., 2009) Twice a year (Rajagopal et al., 1998) or sometimes less frequently (Lee 1985). This therefore puts a large quantity of propagules in the vicinity of a potential vector. In the case of *P. viridis*, a small number of mature individuals may be sufficient for the establishment of a founder population, as demonstrated by McDonald (2012) in western Australia The author also describes the intersection of (potentially) reproducing individuals found on two naval vessels in

			western Australia in 2011. These had resulted in a small founder population inside one of the vessel's sea chests. Larvae have a long-lasting planktonic stage with studies showing settlement times ranging from 20-24 days at 27 ºC and 34- 41days at 24 ºC in hatchery conditions (Nair & Appukuttan 2003). Larvae produce a byssal thread to increase drag, prolonging time spent suspended in the water column and increasing potential for transportation (Baker et al. 2007).
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	Very Likely	High	*P. viridis* is tolerant of rapidly changing environmental conditions and has a long-lasting planktonic larval phase of up to 41 days at 24ºC (Nair & Appukuttan 2003). This would enable transport from ports containing known populations (e.g Kingston Harbour Jamaica) before settlement. For example MSC (2018) provide estimated shipping times of: 37 – 50 days Taiwan to Italy; 30-34 days Jamaica to Italy; 15-27 days USA (known infested locations) to Italy. If resident for 2 or more months in ballast tanks, individuals may become reproductively viable if conditions are appropriate and sufficient suspended food matter is available to promote growth to adulthood.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	likely	Medium	The Ballast Water Management Convention entered into force on the 8th September 2017 and is currently in the process of being implemented. Although it is an existing practice, its current effectiveness of limiting introductions from occurring will be comparatively low in comparison to what it will be once implementation has occurred. The Convention requires all ships in international traffic to manage their ballast water certain standard, according to a ship-specific ballast water management plan. The ballast water management standards are being phased in

			over a period of time. New ships must meet the ballast water treatment standard i.e. have a ballast water treatment system in place. Since the introduction of the IMO voluntary ballast water exchange standard in 1997, existing ships should exchange ballast water mid-ocean as a mitigation method. And existing vessels will also need to meet the ballast water treatment standard by the date of a specified renewal survey. Eventually, most ships will need to install an on-board ballast water treatment system. Both standards would be sufficient to greatly reduce the risk of the introducing *P. viridis*. The ballast water convention will be at different stages of implementation within the RAA depending on the country in question and if/when the convention was ratified by the state. Of those countries identified as having currently suitable climate for invasion in section 1, Bulgaria, Croatia, France Greece, Malta, Portugal and Spain have ratified the convention (as of 06/08/18, IMO). It is however, difficult to determine at what stage of implementation or enforcement of the Convention is at within the different states. By the end of the lead in time for the Convention all countries will need to be applying the convention within scope of the articles of the Convention. Until the Convention has been fully implemented then the risk of the organism surviving passage via this pathway remains likely.
1.7. How likely is the organism to enter the risk assessment area undetected?	Very Likely	High	Larvae and propagules are microscopic and could be overlooked in water. Post-settlement larvae and spat are also very small and would be easily overlooked on visual inspection, especially in confined, accessible areas.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	Medium	Shipping takes place year-round and conditions within potentially habitable parts of the RAA are suitable for development and growth year-round. Arrival during

			warmer, summer months would enhance growth and reproductive rates, enhancing establishment prospects further. Within its native and invaded range, spawning regimes vary with location. Some populations reproduce year-round, whilst others twice a year and in some areas spawning only takes place once a year. McFarland et al. (2016) provide a summary of these differences and themselves found invasive populations in the USA to spawn year-round, including at temperatures as low as 13ºC.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Very Likely	High	Larvae have a long-lasting planktonic larval phase. Larvae produce a byssal thread to increase drag and transport by currents. When ready to settle, this can be on any surface, floating or attached and larvae are able to attach even in fast currents (Rajagopal *et al.*, 2006). If larvae were present and suspended in ballast water as it was released into surrounding waters, they would therefore likely be release and would have a high likelihood of settling.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	Moderately Likely	Medium	While the Ballast Water Convention has come into force, there is still a need to implement, which will be at different stages within the RAA. Until implementation has occurred across the RAA then the risk of introduction is moderately likely.
Pathway name:	**TRANSPORT - STOWAWAY : Ship/ Boat Hull fouling**		
1.3. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	Unintentional	High	*P. viridis* is a known fouling organism capable of attaching to ships' hulls. In 2001 individuals were intercepted in Cairns, Australia and an intensive eradication and monitoring process identified and destroyed a number of individuals nearby (Baker et al., 2007). *P. viridis* is capable of attaching to multiple surface types using byssal threads and is capable of remaining attached in very strong currents (Rajagopal *et al.*, 2006). Wells et al. (2017) provide a summary of

			numerous examples of occasions when *P. viridis* has been introduced and intercepted to Australian waters as part of ship and equipment fouling. These examples include: fishing vessels; construction vessels; naval vessels; bulk cargo vessels; a cruise liner; and private yacht. It is therefore highly likely to attach to vessel hulls in its current range. Many cargo and recreational vessels move from the known range to the RAA daily. The species is considered to be very difficult to control using common antifouling techniques (Rajagopal et al., 2006) and as such, is more likely to be able to attach and remain attached to the hulls of vessels.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	likely	Medium	Mussels are likely to inhabit man-made structures within native and non-native ranges, including harbours and docks. This may be at a density of up to 35,000m^{-2} (Rajagopal 1991). Due to food availability and other favourable conditions, these aggregations are likely to occur close to the surface and within range of vessel hulls. *P. viridis* reach reproductive age rapidly (2-3 months) and are highly fecund broadcast spawners, capable of reproducing throughout the year in suitable conditions (Rajagopal et al., 2006). This therefore puts a large quantity of propagules in the vicinity of a potential vector. Larvae have a long-lasting planktonic phase and produce a byssal thread to increase drag, prolonging time spent suspended in the water column and increasing potential for transportation. They are capable of attaching to a variety of smooth surfaces in fast flowing water, as is demonstrated by their ability to foul cooling pipes in power stations (Rajagopal et al. 1991, 1996; Masilamoni et al., 2002). Their ability to attach to floating objects makes attachment to vessel hulls likely.

1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	High	*P. viridis* is a known fouling organism capable of attaching to ships' hulls. In 2001 individuals were intercepted in Cairns, Australia and an intensive eradication and monitoring process identified and destroyed a number of established individuals nearby (Baker et al., 2007). Individuals may become reproductively viable within 2-3 months of settlement, at which point they have the potential to produce additional offspring in very large numbers, potentially spawning throughout the year (Rajagopal et al., 2006). Additional strong evidence is also presented in McDonald (2012) who describes the intersection of (presumably) reproducing individuals found on two naval vessels in western Australia in 2011. These had resulted in a small founder population inside one of the vessel's sea chests.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	Likely	High	Hull fouling is controlled via anti-fouling paints and cleaning practices both in relation to 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. 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. Therefore, the organism is considered likely to be able to survive passage.

1.7. How likely is the organism to enter the risk assessment area undetected?	Likely	Medium	Large numbers individuals on hulls might be detected on inspection as clumps are conspicuous. However, this would depend on hull inspection as populations would not necessarily be visible from the surface, although this process is not legally required in Europe. Larvae and propagules are microscopic and could be overlooked in water. Post-settlement larvae, spat and sparsely dispersed, fouled, individuals at all stages would be easily overlooked on visual inspection, especially in confined, inaccessible areas.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	Medium	Shipping takes place year-round and conditions within potentially habitable parts of the RAA are suitable for development and growth year-round. Arrival during warmer, summer months would enhance growth and reproductive rates, enhancing establishment prospects further. Within its native range, some populations reproduce year-round, whilst others twice a year (Al-Barwani et al., 2016).
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	Moderately likely	Medium	It has been suggested that low adult body condition results from time spent in open water with reduced food availability. It is believed that this poor condition may restrict spawning (hence transfer) potential during short stops in potential new environments (Huhn et al., 2017, Heersink et al., 2014). Heersink et al. (2014) have connected the two known successful *P. viridis* spawning events to longer dock time coinciding with extreme warming events. If spawning does occur, larvae have a long-lasting planktonic larval phase. Larvae produce a byssal thread to increase drag and transport by currents. When ready to settle, this can be on any surface, floating or attached and larvae are able to attach even in fast currents (Rajagopal et al., 2006).

1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	likely	Medium	In the absence of any formal control measures for this pathway, which has been strongly associated with the introduction of the species elsewhere, then it would seem likely that entry would occur via hull fouling.
Pathway name:	RELEASE IN NATURE: Fishery in the wild		
1.3. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	Intentional	Low	Between 1964 and 1999, *P. viridis* has been intentionally introduced into Southwest China, New Caledonia, Fiji, Tonga, Tahiti, Western Samoa, Japan, Cook Islands and Cape Verde Islands. All but two (Cape Verde and Cook Islands) have resulted in successfully established populations (Baker et al., 2007). This rate of success suggests that in the right conditions intentional introduction is likely to result in the transfer to the natural environment. There is a strong economic incentive to introducing and growing this species, however current legislation in the form of the Alien Species in Aquaculture Regulations (708/2007) prevents the deliberate introduction of non-native species for aquaculture, unless potential risks are mitigated within EU member states. Introduction to northern African countries with different levels of control than the EU may result in spread from these other introduced populations.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	very unlikely	Medium	To establish an aquaculture site within the EU for this species the organism would need to be risk assessed under 708/2007. This is likely to highlight that the species cannot be farmed without extensive mitigating measures (e.g. only farmed in enclosed indoor recirculating systems) put in place making any venture more expensive and therefore less desirable, or altogether impossible. If someone tried to set up a site illegally, then large quantities would need to be bought into the EU live, which would be difficult to achieve.

1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	likely	High	Between 1964 and 1999, *P. viridis* has been intentionally introduced into Southwest China, New Caledonia, Fiji, Tonga, Tahiti, Western Samoa, Japan, Cook Islands and Cape Verde Islands. All but two (Cape Verde and Cook Islands) have resulted in successfully established populations (Baker et al., 2007). This rate of success suggests that in the right conditions intentional introduction is likely to result in the transfer to the natural environment. Larvae have a long-lasting planktonic larval phase. Larvae produce a byssal thread to increase drag and transport by currents. When ready to settle, this can be on any surface, floating or attached and larvae are able to attach even in fast currents. It is therefore likely that any individuals maintained on open systems or grow in the wild in the right conditions could release propagules and transfer the surrounding area.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	Very unlikely	High	If aquaculture of this species was established in North African countries, then currents in the Mediterranean could be expected to carry larvae from North Africa into European waters within the 13-41 day larval lifespan (Rajagopal et al. 2006). Gilg et al. (2014) examined settlement patterns of *P. viridis* in northeastern Florida. They found that most larvae settled within 10 km, but some were at least 18 km from a potential source population. Their model projections suggested that dispersal distance along the open coast could potentially exceed 100 km. If these model predictions are accurate, movement within the Mediterranean from countries outside the RAA if introduced is quite possible. However, this part of the assessment only deals with the deliberate introduction of the species for aquaculture into the RAA, which due to legal restrictions would be very unlikely to occur.

1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	Moderately Likely	High	The predominate risks of entry into the RAA comes from accidental introductions relating to marine traffic (recreational and commercial) through both ballast and hull fouling. The ballast water pathway is being addressed via the Ballast Water Convention, therefore the risk from this particular pathway is likely to be reduced in time. The IMO and its member states are currently working to draft and adopt an instrument to address biofouling. If the BWMC is the model for implementation, entry into force could be many years away.
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	Moderately Likely	Medium	**Future climate:** (50 – 100 years, based on RCP 4.5 and RCP 8.5) As seas become warmer, it is likely that the species will expand its current range into warmer areas and potentially increase the number of potential source populations, increasing the number of shipping routes which could be utilised as a pathway for spread. This will be coupled with an increase in the number of suitable destination sites. It is possible that with warming climate, the introduction of this and other warm water species to the RAA (possibly to replace colder water species currently cultured) will become a more appealing prospect and may lead to an increase in political pressure to allow introduction as well as increasing the risk of illegal introductions and introductions to neighbouring countries outside the EU, which may the spread naturally to the RAA. McDonald (2012) identified spawning and recruitment events in Temperate Western Australian waters from a ship’s hull, which was directly related to a seasonal ‘heat pulse’ raising water temperatures by between 3 and 5 degrees. Such events are likely to increase in frequency with climate change and may result in an

			increased likely hood of escape and transfer of larvae from introduced populations.

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. If the species is established in all Member States, continue with Question 1.16.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	Likely	High	*P. viridis* is capable of colonising a range of substrates and is highly tolerant of varying temperature, salinity and turbidity. Mortality caused by lowest winter sea temperature is likely to be the most important factor controlling the northward spread of *P. viridis* in the RAA. Increasing winter temperature is likely to lead to improved winter survival where temperatures do not drop below 10ºC to 14ºC. Increased summer temperatures will also increase the habitable and optimal range of the species and is likely to enhance reproductive output, even in currently habitable areas. Studies in South Carolina (USA) showed that individuals were able to survive winter conditions where water temperature dropped as low as 10ºC (Knott et al., 2008). Urian et al. (2011) suggest that the critical temperature threshold for survival in *P. viridis* is between 10 and 14 ºC and that this is likely to be a controlling factor in the northward spread of the species in the USA. Gilg et al. (2014) found larval settlement taking place at Spring temperatures as low as 13ºC. It has been suggested that populations in Tampa Bay Florida may be selecting for more cold-tolerant individuals (Benson et al., 2001). It is likely that similar temperature barrier would be

			encountered in current conditions in the north of the Bay of Biscay where temperature drops below 10ºC in Winter and Spring spawning temperature of 13ºC is not met. Exposure to temperatures exceeding 31.5 ºC have been shown to cause high levels of mortality (Nicholson 2002), whilst Segnini de Bravo et al. (1998) found no mortality of individuals exposed to temperatures of 33.5ºC. In the same study. Sea temperatures exceeding this do not occur in the RAA. Temperatures below 6ºC were found to be lethal. However, it is likely based one the Baker et al. (2012) reported a mass mortality, including 100% loss of intertidal populations in Tampa Bay Florida, following freezing air conditions. At the same time, subtidal populations were unaffected and areas where loss had occurred were dominated by *P. viridis* 6 months following the event, suggesting a high recovery potential if subtidal populations remain viable. Firth et al. (2011) found that winter temperatures below 2ºC (down to 0.53ºC) for more than 6 hours of exposure resulted in mass mortality of *P. viridis*. Corroborating the findings of other studies. There is a synergistic effect of temperature and salinity on the survivability of *P. viridis*, with tolerance to salinity change been decreasing at lower temperatures (Yuan et al., 2016). These synergistic impacts are likely to limit the range and potentially habitable areas within the RAA.
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism’s current distribution?	Likely	High	Salinity levels comparable to native range and within know tolerance are also found throughout the RAA. *P. viridis* is euryhaline and studies have shown a broad cardiac tolerance to salinity change

			comfortably tolerating salinities ranging from 15ppt – 35ppt (Nicholson 2002). *P. viridis* does not appear to be capable of tolerating salinities lower than 15ppt and can tolerate short spells of low salinity by closing its valves (McFarland 2013). Where salinity change is gradual *P. viridis* collected in Florida were able to survive (>9%survival) in salinity treatments of 9ppt for 28 days, following acclimatization to a 30ppt treatment (McFarland et al., 2014). Studies have show that *P. viridis* is able to tolerate salinities of up to 80ppt with 50% mortality with an optimum salinity for growth between 27 and 65ppt (Sivalingam 1977). These levels suggest that *P. viridis* would be able to tolerate and even thrive at higher salinity levels (>39ppt) found in parts of the Mediterranean. Exposure to air, both in hot (McFarland et al., 2014) and cold (Power et al. 2004, Baker et al. 2012) conditions can lead to mortality and it is likely therefore that populations will be limited to subtidal areas and locations within the RAA with small tidal ranges (such as the Mediterranean). *P. viridis* is capable of accumulating and tolerating relatively high levels of heavy metals, including copper (Chan 1988). Although long-term chronic exposure to copper has been shown to adversely impact body functions, including growth (Sze & Shing 2000) It also has a high tolerance for anoxic conditions (Wang et al., 2005, 2011). Both of these traits may help the species become established in areas of poor water quality (e.g. harbours, marinas, industrial areas and ports) within the RAA. Heersink et al. (2014) investigated introductions of *P. viridis* to Australia, estimating that thousands of

			introductions had taken place, with only two recorded spawning events. Known species tolerances and optimal conditions in invaded and native range appear to match those of the area they studied. The authors therefore suggest that an alternative factor is playing a strong role in controlling the establishment of the species. Several reasons are suggested including water chlorophyll levels. Huhn et al. (2017) suggest that mussels living in fouling communities on ship's hulls may have reduced condition and reproductive potential as a result of exposure to oligotrophic conditions experienced during passage over open ocean. Such conditions occur in parts of the Mediterranean and on shipping routes from outside the RAA and therefore may impair the ability of these introduced individuals to become established if introduced. There is a synergistic effect of temperature and salinity on the survivability of *P. viridis*, with tolerance to salinity change been decreasing at lower temperatures (Yuan et al., 2016). These synergistic impacts are likely to limit the range and potentially habitable areas within the RAA.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the RAA?	Widespread	High	*P. viridis* Is capable of colonising and living on a wide range of substrates, including man-made and natural hard structures and soft sediment areas. It is also capable of colonising drifting and floating objects and ropes, and other man-made objects. From intertidal habitat to a depth of approximately 42 m. These habitat conditions are wide spread throughout the RAA and overlap with regions of suitable climate and other abiotic conditions.

1.16. 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 ?	NA	High	There is no evidence that any other species is critical to the survival of *P. viridis.*
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	Likely	Medium	*P. viridis* is a highly fecund, fast growing species, tolerant of a wide range of environmental conditions. These features mean that it is able to capitalise on environmental change and events which may displace other species. It is also capable of attaching to and overgrowing existing organisms. In studies, *P. viridis* has outcompeted its congener *P. perna* (e.g. Segnini de Bravo et al., 1998). The same species (previously assigned a different species *P.picta*) is present in the Mediterranean. Prabha et al. (1998) identified a number of invertebrate species native to India, which produced chemicals which inhibited development of byssal threads and attachment in *P. viridis*. Soft corals, sponges and a species of bivalve were all found to produce effective ‘antifoulants’ which prevented attachment. Other studies have identified additional species of Gorgonian coral and sponge with similar qualities (Wilsanand et al., 1999). The findings suggest that there may be species in the RAA capable of resisting colonisation by the mussel and is an area worthy of further study. McFarland et al. (2016), studying the reproductive strategy of invading populations of *P. viridis* in the USA, identified year-round gamete production and high reproductive potential. The authors have suggested that this trait could give the mussel a

			competitive edge over the native oyster species. The authors do however also state that these traits vary greatly between geographical locations. Whilst comparative conditions exist in the south and south eastern parts of the Mediterranean, other regions have differing conditions that may result in different and as yet unknown reproductive strategies, which may or may not increase competition with native and farmed oysters in the region. Yamada et al. (2009) cite studies (in Japanese), which have shown a decline in *Mytilus galloprovincialis* , - which is native to the RAA but invasive in Japan - in Japanese waters. In some cases these seem to have been replaced by *P. viridis*. There is currently little evidence to suggest *P. viridis* competitively eliminated *M. galloprovincialis*, however its presence and dominance of suitable substrate would make recolonization unlikely. The authors suggest the change may have been a result of warming sea conditions or pressure from native parasites, but causation is not clear and would require further study.
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	Moderately Likely	Medium	*P. viridis* is a highly fecund, fast growing species, tolerant of a wide range of environmental conditions. It is therefore capable of recovering from pressures exerted by other species. In the RAA, a number of potential predators exist, these include: Starfish, predatory gastropods, fish, seabirds, crabs and lobsters. These species have adapted to feed on mytilids and other native mussel species and it is likely that they would also be able to take *P. viridis* as a prey species.

			Many similar predators exist within the natural and introduced range and given the right conditions, it has become established. *P. viridis* utilises number of anti-predator behavioural traits including increased byssal thread production and clumping behaviour (Cheung et al. 2004, Wang et al. 2013). It has been suggested that one reason why *P. viridis* has failed to become established in Australia despite thousands (Heersink et al., 2014, Wells 2017) of introductions is the presence of a large number of potential predatory species. Predation combined with sub-optimal conditions may reduce the ability to become established. *P. viridis* is able to adapt its shell morphology due to the presence of predatory species, potentially resulting in reduced predation (Cheung et al. 2004). Pea crabs have been found within *P. viridis* during studies in Malaysia (Al-Barwani et al., 2011) and have been found to inhabit and impair the condition of *P. viridis* in Japan (Yamada et al., 2009) and India (Jose & Deepthi 2005). These commensal parasites are known to reduce the condition of mussels and reduce reproductive potential. However, to what degree European pea-crab species will inhibit establishment is unknown. Mass mortality events have been observed in *P. viridis* as a result of blooms of the toxic dinoflagelate *Karenia brevis* (McFarland et al., 2015, Baker et al., 2012). This species has been recorded in the Mediterranean and North East Atlantic alongside its congener *K.mikimotoi*. However mass mortalities were not observed during blooms of *Pyrodinium bahamense* which produces saxotoxin (Baker et al., 2012).

			Skein et al. (2018) studied the interactions between two predators of bivalves with two introduced mussel species and found that the predatory starfish and lobster fed preferentially on native species of mussel, preferring them to the non-native mussel species. Such experiments highlight the potential for introduced species to escape predation by unfamiliar predatory species. To the best of the authors knowledge there has been no detailed assessment of the disease profile of this species within its native or introduced range. It is therefore impossible to make comment on what diseases movement of this species may carry and how these may have further impact on the ecosystem it is introduced into. The lack of disease profiling of invasive/horizon species has been highlighted on multiple occasions (e.g. Roy et al. 2017).
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	likely	Medium	To the best of the author's knowledge there are few/no specific management practices in place in the RAA that would prevent the establishment of this species. However, several countries within the area have rapid detection and response processes established. If such processes result in early detection of *P. viridis*, then methods previously applied - for example in Australia - to control the species could be utilised. The likelihood of success does however rely on the introduction being detected early in the invasion process and sufficient resources being made available to implement the controls.
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	Moderately likely	Medium	In water hull cleaning, which is conducted on both commercial and recreational vessels, would facilitate the removal and transplantation of

			fouling organisms. Although the chances of the organism surviving if dislodged in appropriate conditions is not known, this practice is common in the RAA.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	Moderately Likely	High	Thermal treatments: *P. viridis* is relatively tolerant of high temperatures, for short periods of time. However, based on the work of Firth et al. (2011) emmersed exposure to temperatures lower than 2ºC for 6 hours or more would be a potential method of eradication from fouled vessels and equipment.. Air drying: Not tolerant of emersion, so may be effective. Chemical treatment: Tolerant of higher chlorination levels than many fouling species (Rajangopal et al., 2006), however treatment at high levels over prolonged period may be effective. Additionally, *P. viridis* is capable of accumulating and tolerating relatively high levels of heavy metals, including copper, which is often used to control fouling organisms (Chan 1988). Features which may inhibit successful control efforts include microscopic, long-living mobile larval phase, tolerance to rapidly changing environmental conditions and anoxia, plastic morphology able to adapt to varying conditions, rapid maturation of individuals and high fecundity, ability to colonise a range of often cryptic and inaccessible habitats.
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	Very Likely	High	*P. viridis* reach reproductive age rapidly (2-3 months) and are highly fecund broadcast spawners, with females capable of releasing 3 to 6 X 10^6 (3-6 million) Or rarely 19 × 10^6 (19 million)

			eggs in a single spawning event (McFarland et al., 2016). *P. viridis* spawning may take place year round or in biannual cycles, depending on location. This is likely dependent on environmental parameters combined with availability of food. The phenomenon of year-round reproduction is likely to take place in areas where conditions are stable throughout such as tropical equatorial regions (Al-Barwani et al., 2011, Rajagopal et al., 2006). Such stable conditions may exist artificially within the RAA, for example in and around the cooling effluents produced by power stations. In warm, but less stable environments spawning appears to be restricted to warmer months with two (sometimes one) annual peaks (Shafee 1989, Gilg et al., 2014, McFarland et al., 2014). Gilg et al. (2014) found that spawning events were most closely correlated with temperature change than a specific temperature and in particular that spawning and settlement peaks occurred as a result of a warming trend occurring approximately 2 months following the coldest winter temperature. The authors found settlement indicating spawning at a wide range of temperatures over a 3 year period. Al-Barwani et al. (2011) identified that male: female sex ratios are usually 1:1 and that such a structure would enhance the reproductive success during synchronised broadcast spawning. Spawning in *P. viridis* can be induced by the presence of conspecifics nearby and drops in salinity. It can be initiated by males or females who release two streams of gametes into the water for external fertilisation. (Stephen & Shetty 1981).

			Hermaphroditism has been observed on occasion although this is considered a very rare phenomenon (Al-Barwani et al., 2011).
1.23. How likely is the adaptability of the organism to facilitate its establishment?	Moderately Likely	High	*P. viridis* is a highly adaptable species, able to adapt to a range of temperatures and salinity change (Rajagopal et al., 2006). The species is also able to adapt its shell morphology due to the presence of predatory species, potentially resulting in reduced predation (Cheung et al. 2004). Goh & Lai (2014) have conversely identified that although *P. viridis* can tolerate a range of thermal conditions, their optimal temperature range may in fact be limited. Non-fatal impacts of temperature increases may be sufficient to inhibit growth and other functions, thus restricting establishment and spread in some warmer areas and as temperature increases over time.
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	Likely	Medium	Populations have become established throughout its range despite initial low genetic diversity and there is currently no evidence to suggest that this would not be the same within the RAA. A study by Gobin et al. (2014) identified a very low genetic diversity in Trinidad and Tobago populations 20 years after introduction and the authors suggest that this may be a reason for the species' patchy distribution in the region. Additionally, Tampa Bay specimens had the highest genetic similarity with specimens from Trinidad (Benson et al., 2001)
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	Likely	Medium	Between 1964 and 1999, *P. viridis* has been intentionally introduced into Southwest China, New Caledonia, Fiji, Tonga, Tahiti, Western Samoa, Japan, Cook Islands and Cape Verde Islands. All but two (Cape Verde and Cook Islands) have resulted in successfully established

			populations. Accidental or undetermined introductions to Hong Kong, Taiwan, Japan, Trinidad, Jamaica, Venezuela and Florida all resulted in establishment. This rate of success suggests establishment would be likely once introduced to a suitable habiat. An introduction in Cairns, Australia was intercepted and presumed to have been eradicated. (Baker et al., 2007). Heersink et al. (2014) report on multiple additional Australian interceptions and calculate that *P. viridis* is likely to have arrived into apparently suitable locations in Australia thousands of times in the past 50 years. However it has still not successfully established, suggesting another as yet unknown inhibiting factor. It is not currently known whether the unknown inhibiting factor postulated by Heersink et al. (2014) and indeed other as yet unidentified factors might have in the RAA and further research is required. One possible limiting factor suggested by the authors was low chlorophyll a levels. This theory is further supported by a correlation between lower/ less regular chlorophyll a levels and lower body condition index in *P. viridis* examined from two sites in Malaysia (Al-Barwani et al., 2016). And is particularly relevant for the oligotrophic waters of the Mediterranean, where suitable climatic conditions are most likely to be encountered
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur?	Likely	Medium	Unless the pathway of origin is stopped or cut-off, introductions would likely continue and casual populations would be very likely to occur. However, unless populations become established, impacts of small numbers of individual animals will likely be minimal.

1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	Likely	Medium	***Marine regions:*** North-east Atlantic Ocean, Mediterranean Sea, Black Sea (far west) ***Marine subregions:*** Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea and Central Mediterranean Sea, Aegean-Levantine Sea. **Explanation** Whilst minimum summer temperatures do not currently fall within the optimal range of 26 – 32ºC described by Urian et al. (2011) in any part of the RAA. Maximum summer temperatures in the Tyrrhenian, Ionian & Levantine basins do currently fall within this optimal temperature range. Current annual temperature ranges throughout the Mediterranean and the coasts of Portugal and Atlantic Coast of Spain and to the northern Bay of Biscay fall within the range of temperatures *P. viridis* experiences in its native range, which are between 12 and 32ºC (Urian et al., 2011). Salinity levels comparable to native range and within know tolerances are also found throughout these areas. In the Eastern Black Sea, salinity levels and winter temperatures are lower than those required for the survival of *P. viridis*. The western region of the Black Sea is currently within habitable ranges and establishment might be possible here. Salinity and temperature in the Baltic are not currently suitable to sustain populations. Mortality caused by lowest winter sea temperature is likely to be the most important factor controlling

			the northward spread of *P. viridis* in the RAA. However, the entire Mediterranean currently remains at a temperature tolerable to *P. viridis* throughout even coldest recorded winters. The Black Sea and sea areas north of the Bay of Biscay currently drop below the 10ºC threshold during winter, suggesting the areas would not be suitable for the species' survival in current conditions. *P. viridis* is capable of colonising a range of substrates and is highly tolerant of varying temperature, salinity and turbidity.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	Likely	Low	**Future climate:** (50 – 100 years, based on RCP 4.5 and RCP 8.5) ***Marine regions:*** North-east Atlantic Ocean, Mediterranean Sea, Black Sea ***Marine subregions:*** English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea. ***Explanation*** Based on future warming scenarios (0.6 – 2.5ºC in 50 years and 1.4 – 5.8ºC in 100 years), all parts of the Mediterranean and the Atlantic Coast of Europe from Spain to the northern Bay of Biscay could fall into the optimum temperature range of 26-32oC described by Urian et al. (2011) within the next 50 to 100 years. It is possible that this suitable habitat will extend as far as the South

			West English Channel and Lower Celtic Sea (French coast) but this is less likely. Mortality caused by lowest winter sea temperature is likely to be the most important factor controlling the northward spread of *P. viridis* in the RAA. Increasing winter temperature is likely to lead to improved winter survival where temperatures do not drop below 10ºC to 14ºC and increased spring temperatures are likely to enhance spawning capabilities. In future predicted warming scenarios, the area habitable to *P. viridis* may move northwards in the next 50-100 years to include some parts of the Western English Channel. Increased summer temperatures will also increase the habitable and optimal range of the species and is likely to enhance reproductive output, even in currently habitable areas. Future predicted warming and increased salinity associated with reduced freshwater input may extend the potential habitable area of the Black Sea. If salinity decreases in the Baltic as models predict, the area will continue to be unable to support *P. viridis* populations in the future.

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	Major	Medium	**Larval dispersal** *P. viridis* has a planktonic larval phase. The length of larval phase has been found to vary greatly and the results of several in culture studies have been summarised by Baker et al. (2007) with different studies reporting observed larval phases ranging from 8-12, 15-18 and 24-19 days. The authors also suggest a planktonic post-larval phase observed in similar species may be possible, but has not yet been observed in *P. viridis*. Larvae secrete byssal threads, which facilitate spread by reducing sinking and enhancing drag, making transportation by currents more effective (Rajagopal et al., 2006). These threads are also used to attach to a variety of substrates and facilitate attachment even in very strong currents. Depending on prevailing currents in the area of introduction, larval dispersal over a long distance, leading to spread is highly likely. **Rafting on natural debris** The byssal threads created by planktonic and post-settlement stage larvae, combined with a presence high in the water column enable attachment to a range of different floating substrates (S.t.A. Buddo et

			al., 2003, Baker et al., 2007). Debris such as drift-wood can travel great distances on ocean currents and would be capable of transporting and spreading reproductively viable mussels within the RAA
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	Major	Medium	**Transport – Stowaway: Ship / Boat Hull fouling** *P. viridis* is a fouling organism, capable of attaching to boats and mobile structures and equipment. It is therefore highly likely that human vectors will play an important role in spreading the species should it arrive. Baker at al (2007) have suggested that much of the species' current range could have been due to historic human spread. Several studies (for example Knott et al., 2008, Huhn et al., 2015) have identified populations with the potential to become reproductively viable - particularly if dislodged in suitable conditions - as fouling organisms on the bottom of cargo and passenger vessels. These vessels were not undertaking international travel, but potentially acting as vectors of further spread within the invaded range. In Indonesia, there is evidence of transportation of *P. viridis* on the hulls of regular passenger ferries (Huhn et al., 2015). Many similar regular ferries operate in the RAA, in particular, between islands in the Mediterranean. These crossings would likely facilitate the species spread. Recreational vessels travelling between ports in the RAA would also provide a potential vector of spread in the area. **Transport – Stowaway: Machinery / Equipment** Wells et al. (2017) describe the introduction of *P. viridis* to Australia on dredges used in construction.

			Such large scale equipment is transported world-wide for specialist development projects. It is also conceivable that individuals may grow on or become entangled in or attach to equipment associated with marine activities (fishing, renewable energy, recreational activities) and that this may be transferred between locations in the RAA, contributing to spread. **Corridor: Attachment to anthropogenic debris and unaided drift** The byssal threads created by planktonic and post-settlement stage larvae, combined with a presence high in the water column enable attachment to a range of different floating substrates (Baker et al., 2007). Debris such as rope, plastic, lost shipping and fishing equipment can travel even greater distances on ocean currents than natural debris and would be capable of transporting and spreading reproductively viable mussels within the RAA. This potential pathway has been described under the 'Corridor' heading following consideration and discussion. It is considered by the authors that the continuous/ regular depositing of debris and its subsequent continuous unaided flow with ocean currents is most analogous to a 'corridor' in the absence of a more appropriate pathway type. **Transport – Stowaway: Ship / Boat Ballast Water** Many of the unintentional introductions globally have been attributed to ballast water. For example, *P. viridis* in Tampa Bay Florida is believed to have been introduced as larvae via ballast water transfer and a population is now well established in the area (Rajagopal et al., 2006). There is evidence to suggest

			that the successful Introduction of *P. viridis* to Jamaican waters was via ship ballast water introduction of larvae (S.t.A. Buddo et al., 2003). There are large amounts of ship (cargo and passenger) movement across the Mediterranean region and many of these vessels undertake ballast water exchange in coastal waters, including habitats suitable for mussel colonisation. It should be noted that throughout the reviewed literature, no evidence other than circumstantial evidence could be found to support the theory that introductions have been through ballast although I is considered the most likely pathway in the cases described.
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	1. Transport – Stowaway: Ship / Boat Hull fouling 2. Transport – Stowaway: Machinery / 3. Equipment 4. Corridor (?): Attachment to anthropogenic debris and unaided drift 5. Transport – Stowaway: Ship / Boat Ballast Water		
Pathway name:	**Unaided: Natural Dispersal (2.1)**		

2.3a. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	Unintentional	High	(includes natural dispersal of larvae and propagules and by attachment to natural flotsam)
2.4a. 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?	Likely	Medium	**Larval dispersal** *P. viridis* has a planktonic larval phase. The length of larval phase has been found to vary greatly and the results of several in culture studies have been summarised by Baker et al. (2007) with different studies reporting observed larval phases ranging from 8-12, 15-18 and 24-19 days. The authors also suggest a planktonic post-larval phase observed in similar species may be possible, but has not yet been observed in *P. viridis*. Larvae secrete byssal threads, which facilitate spread by reducing sinking and enhancing drag, making transportation by currents more effective (Rajagopal et al., 2006). These threads are also used to attach to a variety of substrates and facilitate attachment even in very strong currents. Depending on prevailing currents in the area of introduction larval dispersal over a long distance, leading to spread is highly likely. The distance of spread achievable would be dependent on prevailing currents in the area of introduction and this would vary by site. Gilg et al. (2014) for example found that in Forida, larvae most often travelled and settled at distances of 10km or less, but occasionally that spat could be found 18km from the source. Model predictions by the authors however suggest that a dispersal distance of >100km could often occur. Such dispersal potential would make spread with the RAA rapid. At early life stages, *P. viridis* is very small and difficult to see with the naked eye. Identification of microscopic Mussel larvae and post-larvae to species level requires very specialist knowledge and even if plankton monitoring were

			taking place, there would be a low chance for most of successfully detecting *P. viridis*. **Rafting on natural debris** The byssal threads created by planktonic and post-settlement stage larvae, combined with a presence high in the water column enable attachment to a range of different floating substrates (Buddo et al., 2003, Baker et al., 2007). Debris such as drift-wood can travel great distances on ocean currents and would be capable of transporting and spreading reproductively viable mussels within the RAA Mussels are likely to inhabit man-made structures within including harbours and docks. Due to food availability and other favourable conditions, these aggregations are likely to occur close to the surface and within range of floating objects. Green mussels reach reproductive age rapidly (2-3 months) and are highly fecund broadcast spawners, capable of reproducing throughout the year in suitable conditions (Rajagopal et al., 2006). This therefore puts a large quantity of propagules in the vicinity of a potential vector.
2.5a. How Likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	Likely	High	*P. viridis* is tolerant of rapidly changing environmental conditions and has a long-lasting planktonic larval phase. If resident for 2 or more months, individuals may become reproductively viable if conditions are appropriate and sufficient suspended food matter is available to promote growth to adulthood.
2.6a. How likely is the organism to survive existing management practices during spread?	Very Likely	High	There are currently no known management practices within the RAA that would impact species movement attached to natural floating debris.

2.7a. How likely is the organism to spread in the risk assessment area undetected?	Very Likely	Medium	The similarity to native Mediterranean mussel species, especially *P.perna* may lead to misidentifications and result in invasions going undetected. At early life stages, *P. viridis* is very small and difficult to see with the naked eye. Identification of microscopic Mussel larvae to species requires very specialist knowledge and even if plankton monitoring were taking place, there would be a low chance for most of successfully detecting *P. viridis*.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	Likely	Medium	Heersink et al. (2014) investigated introductions of *P. viridis* to Australia, estimating that thousands of introductions had taken place, including an unknown but potentially high number attached to discarded fishing gear. The introductions resulted in only two recorded spawning events. Known species tolerances and optimal conditions in invaded and native range appear to match those of the area they studied. The authors therefore suggest that an alternative factor is playing a strong role in controlling the establishment of the species. Several reasons are suggested including water, chlorophyll levels. One suggestion is that the stress associated with spending time in oligotrophic open water may decrease condition and inhibit spawning during short stop-overs. Huhn et al. (2015) suggest a similar phenomenon on passenger ferries in Indonesia.
2.9a. Estimate the overall potential for spread within the Union based on this pathway?	Rapidly	Medium	Based on the life history traits discussed (high fecundity, long lasting, pelagic larval stage etc) combined with its ability to colonise a range of substrates, and tolerance of changing environmental conditions.
End of pathway assessment, repeat as necessary.			

Pathway name:	**Transport Stowaway: ship/boat hull fouling**		
2.3b. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	Unintentional	High	*P. viridis* is a known fouling organism capable of attaching to ships' hulls. In 2001 individuals were intercepted in Cairns, Australia and an intensive eradication and monitoring process identified and destroyed a number if established individuals nearby (Baker et al., 2007). *P. viridis* is capable of attaching to multiple surface types using byssal threads and is capable of remaining attached in very strong currents (Rajagopal *et al.*, 2006). It is therefore highly likely to attach to vessel hulls in its current range. move from the known range to the RAA daily. The species is considered to be very difficult to control using common antifouling techniques (Rajagopal et al., 2006) and as such, is more likely to be able to attach and remain attached to the hulls of vessels. In Indonesia, there is evidence of transportation of *P. viridis* on the hulls of regular passenger ferries (Huhn et al., 2015). Many similar regular ferries operate in the RAA, in particular, between islands in the Mediterranean. These crossings would likely facilitate the species spread. Recreational vessels travelling between ports in the RAA would also provide a potential vector of spread in the area. It is also possible that fishing vessels operating from areas inhabited by *P. viridis* or fishing over *P. viridis* beds might act as vectors through hull and gear fouling or release of incidental by-catch containing mussels.
2.4b. 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?	Very Likely	High	Mussels are likely to inhabit man-made structures within including harbours and docks. Due to food availability and other favourable conditions, these aggregations are likely to occur close to the surface and within range of vessel hulls. Green mussels reach reproductive age rapidly (2-3 months) and are highly

			fecund broadcast spawners, capable of reproducing throughout the year in suitable conditions (Rajagopal et al., 2006). This therefore puts a large quantity of propagules in the vicinity of a potential vector. Larvae have a long-lasting planktonic phase and produce a byssal thread to increase drag, prolonging time spent suspended in the water column and increasing potential for transportation. In the case of *P. viridis*, a small number of mature individuals may be sufficient for the establishment of a founder population, as demonstrated by McDonald (2012) in western Australia The author also describes the intersection of (potentially) reproducing individuals found on two naval vessels in western Australia in 2011. These had resulted in a small founder population inside one of the vessel's sea chests. They are capable of attaching to a variety of smooth surfaces in fast flowing water, as is demonstrated by their ability to foul cooling pipes in power stations. Their ability to attach to floating objects makes attachment to vessel hulls likely.
2.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	Likely	High	*P. viridis* is a known fouling organism capable of attaching to ships' hulls. In 2001 individuals were intercepted in Cairns, Australia and an intensive eradication and monitoring process identified and destroyed a number if established individuals nearby (Baker et al., 2007). Individuals may become reproductively viable within 2-3 months of settlement, at which point they have the potential to produce additional offspring I very large numbers
2.6b. How likely is the organism to survive existing management practices during spread?	Likely	High	Hull fouling is controlled via anti-fouling paints and cleaning practices both in relation to the commercial and recreational sectors. In contrast to ballast water, there are currently no specific conventions or legally binding international frameworks to control bio-

			fouling. 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. 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. Therefore, the organism is considered likely to be able to survive passage.
2.7b. How likely is the organism to spread in the risk assessment area undetected?	Moderately Likely	High	Large individuals on hulls might be detected on inspection as clumps are large. However, this would depend on hull inspection as populations would not necessarily be visible from the surface. Wells et al. (2017) provide a summary of numerous examples of occasions when *P. viridis* has been introduced and intercepted to Australian waters as part of ship and equipment fouling. These examples include: fishing vessels; construction vessels; naval vessels; bulk cargo vessels; a cruise liner; and private yacht. It is therefore highly likely to attach to vessel hulls in its current range. However, the authors note that this interception is due to a focused mitigation initiative, targeting vessels deemed to be of risk and that many other vessels may be arriving with *P. viridis* attached undetected. Such an initiative is not currently established in the RAA and as such the risk of non-detection is event higher. The similarity to native Mediterranean mussel species, especially *P.perna* may lead to misidentifications and result in invasions going

			undetected. Unless forming large clumps, individuals may also be difficult to see amonge foulding communities. At early life stages, *P. viridis* is very small and difficult to see with the naked eye. Identification of microscopic Mussel larvae and post-larvae to species level requires very specialist knowledge.
2.8b. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	Moderately Likely	Medium	Heersink et al. (2014) investigated introductions of *P. viridis* to Australia, estimating that thousands of introductions had taken place, with only two recorded spawning events. Known species tolerances and optimal conditions in invaded and native range appear to match those of the area they studied. The authors therefore suggest that an alternative factor is playing a strong role in controlling the establishment of the species. Several reasons are suggested including water, chlorophyll levels. One suggestion is that the stress associated with spending time in oligotrophic open water may decrease condition and inhibit spawning during short stop-overs. Huhn et al. (2015) identified a similar phenomenon on passenger ferries in Indonesia.
2.9b. Estimate the overall potential for spread within the Union based on this pathway?	Likely	High	*P. viridis* is a fouling organism, capable of attaching to boats and mobile structures and equipment. It is therefore highly likely that human vectors will play an important role in spreading the species should it arrive. Baker at al (2007) have suggested that much of the species' current range could have been due to historic human spread. Numerous studies (Stafford et al., 2007, Knott et al., 2008, Huhn et al., 2015) have identified reproductively viable populations as fouling organisms on the bottom of cargo and passenger vessels, not undertaking international

			travel, but potentially acting as vectors of further spread within the invaded range. In Indonesia, there is evidence of transportation of *P. viridis* on the hulls of regular passenger ferries (Huhn et al., 2015). Many similar regular ferries operate in the RAA, in particular, between islands in the Mediterranean. These crossings would likely facilitate the species spread. Recreational vessels travelling between ports in the RAA would also provide a potential vector of spread in the area. Studies have found individuals fouling motors and hulls of recreational vessels, as well as marina pontoon structures (see for example Knot et al., 2008). Transport of goods and passengers around Europe and between countries and islands in the Mediterranean. Many of these vessels (in particular passenger ferries) have only short docking times and as a result, opportunity for settlement may be reduced.
Pathway name:	**Transport Stowaway: Ship. Boat Ballast**		
2.3c. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	Unintentional	High	
2.4c. 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?	Likely	High	Mussels are likely to inhabit man-made structures, including harbours and docks. Due to food availability and other favourable conditions, these aggregations are likely to occur close to the surface and within range of vessel hulls and ballast inlets. Green mussels reach reproductive age rapidly (2-3 months) and are highly fecund broadcast spawners, capable of reproducing throughout the year in suitable conditions (Rajagopal et al., 2006). This therefore puts a large quantity of propagules in the vicinity of a potential vector. Larvae have a long-

			lasting planktonic phase and produce a byssal thread to increase drag, prolonging time spent suspended in the water column and increasing potential for transportation
2.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	Likely	High	*P. viridis* is tolerant of rapidly changing environmental conditions and has a long-lasting planktonic larval phase. If resident for 2 or more months, individuals may become reproductively viable if conditions are appropriate and sufficient suspended food matter is available to promote growth to adulthood.
2.6c. How likely is the organism to survive existing management practices during spread?	Likely	Medium	The Ballast Water Management Convention entered into force on the 8th September 2017 and is currently in the process of being implemented. Although it is an existing practice, its current effectiveness of limiting introductions from occurring will be comparatively low in comparison to what it will be once implementation has occurred. The Convention requires all ships in international traffic to manage their ballast water certain standard, according to a ship-specific ballast water management plan. The ballast water management standards are being phased in over a period of time. New ships must meet the ballast water treatment standard i.e. have a ballast water treatment system in place. Existing ships should exchange ballast water mid-ocean, but they will need to meet the ballast water treatment standard by the date of a specified renewal survey. Eventually, most ships will need to install an on-board ballast water treatment system. Both standards would be sufficient to greatly reduce the risk of the introducing *P. viridis*. The ballast water convention will be at different stages of implementation within the RAA depending

			on the country in question and if/when the convention was ratified by the state. Of those countries identified as having currently suitable climate for invasion in section 1, Bulgaria, Croatia, France Greece, Malta, Portugal and Spain have ratified the convention (as of 06/08/18, IMO). It is however, difficult to determine at what stage of implementation or enforcement of the Convention is at within the different states. By the end of the lead in time for the Convention all countries will need to be applying the convention within scope of the articles of the Convention. Until the Convention has been fully implemented then the risk of the organism surviving passage via this pathway remains likely.
2.7c. How likely is the organism to spread in the risk assessment area undetected?	Moderately Likely	High	The similarity to native Mediterranean mussel species, especially *P.perna* may lead to misidentifications and result in invasions going undetected. At early life stages, *P. viridis* is very small and difficult to see with the naked eye. Identification of microscopic Mussel larvae to species requires very specialist knowledge and even if plankton monitoring were taking place, there would be a low chance for most of successfully detecting *P. viridis.* The relative lack of monitoring for marine non-native species within the RAA would also limit the ability to detect the species.
2.8c. How Likely is the organism to be able to transfer to a suitable habitat or host during spread?	very Likely	High	Larvae have a long-lasting planktonic larval phase. Larvae produce a byssal thread to increase drag and transport by currents. When ready to settle, this can be on any surface, floating or attached and larvae are able to attach even in fast currents. (Rajagopal *et al.*, 2006). If larvae were present and suspended in ballast water as it was released into surrounding waters, they

			would therefore likely be release and would have a high likelihood of settling.
2.9c. Estimate the overall potential for spread within the Union based on this pathway?	Rapidly	High	Transport of goods and passengers around Europe and between countries and islands in the Mediterranean in particular is common and ballast water exchange occurs from these vessels frequently.
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	Very Difficult	High	Once established, the life history traits discussed- High fecundity; broadcast spawning; long-lived planktonic larvae; ability of larvae to attach to a range of mobile substrate - would make containment extremely difficult. The nature of subtidal marine environment – Cryptic; inaccessible; highly connected; heterogonous – means that containment would be impossible unless populations are in extremely limited, enclosed or semi-enclosed sites. When containment may be possible.
2.11. Estimate the overall potential for spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	Very Likely	High	Once established, the life history traits discussed- High fecundity; broadcast spawning; long-lived planktonic larvae; ability of larvae to attach to a range of mobile substrate - would make spread rapid and very likely. Such spread would occur naturally and follow prevailing currents, where larvae may be capable of travel over long distances. The presence of multiple pathways in the RAA (recreational and commercial shipping; fisheries operations; movement of marine structures and equipment) and limited control of movement between member states would make anthropogenic spread likely within the area.
2.12. Estimate the overall potential for spread in relevant biogeographical regions in foreseeable climate change conditions	Very Likely	Medium	As sea temperatures increase, the extent of habitat available to *P. viridis* will increase. Movements as described in 2.11 would be likely to facilitate successful spread within previously less favorable parts of the Mediterranean. Prevailing currents in the

			Straits of Gibralter would make natural movement of larvae into the Atlantic less likely, but human mediated spread into parts of the Spanish, French and Portuguese Atlantic coast would become more likely and would be accelerated by warming conditions.

MAGNITUDE OF IMPACT			
Important instructions: • Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**
Biodiversity and ecosystem impacts			
2.13. How important is 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?	Major	Low	Studies undertaken by Lopeztegui - Castillo et al. (2014) suggest that the structural complexity provided by low density (156ind/m^2) *P. viridis* aggregations in Cuba have the potential to enhance biodiversity, by providing food and refuge for a wide variety of invertebrate species. However, this and similar work from Venezuela cited therein did not sample uninvaded sites to be able to offer a reliable comparison. It is therefore possible but not certain that similar effects may occur should *P. virdis* become established in the RAA. Mussels including *Perna sp* are known to consume plankton in high quantities (Zeldis et al., 2004). There is potential for this predation to impact open water and coastal trophic interactions and that this might have further impacts on biodiversity. For example such impacts have the potential to impair food and larval supply for filter-feeding species, including habitat forming bivalves and polychaetes, which are important

			ecosystem engineers throughout the RAA, although these impacts do not appear to have been directly studied yet. When undertaking studies of *P. viridis* populations in New Mexico, Baker at al (2007) found that *P. viridis* growing over dead individuals on *Crassostrea virginica* reefs, but no direct evidence that the presence of mussels had caused oysters to die off. This and other circumstantial e.g Baker et al (2003) evidence suggests that further studies should focus on whether *P. viridis* might adversely impact other reef forming organisms within the RAA. Such reef-forming animals create vital habitat for a wide range of native organisms. *P. viridis* forms dense aggregations, with individuals adhering to one-another using byssal threads forming a turf over reefs and other substrates (Rajagopal et al., 2006). This turf may be at a density of up to 35,000m^{-2} (Rajagopal 1991) but more commonly appear to be at maximum densities of 4-10,000 m^{-2} (Baker et al., 2007, McFarland et al., 2014). Such dense turfs have the potential to smother existing species and alter the substrate available to native species. *P. viridis* has the potential to outcompete other fouling species causing changes in community structure and trophic relationships. The magnitude of these impacts does warrant further investigation as the subject seems to be currently understudied, especially in areas comparable to the RAA. *P. viridis* is known to colonise seagrass beds in high densities (Baker et al., 2012, Rajagopal et al., 2006, S.t.A. Buddo et al., 2003). Although the impact of such colonisation is not currently known, given the high conservation importance of seagrass habitat within the RAA, this is an area requiring further study.

			There is some evidence to suggest *P. viridis* has driven out its congener *P.perna* –another bed-forming mussel- from its natural habitat in its invaded range, altering community structure within the area (Segnini de Bravo et al., 1998). However, it is possible that the findings may in fact be a demonstration of habitat segregation between the two species. Yamada et al. (2009) cite studies (in Japanese), which have shown a decline in *Mytilus galloprovincialis* , - which is native to the RAA but invasive in Japan - in Japanese waters. In some cases these seem to have been replaced by *P. viridis*. There is currently little evidence to suggest *P. viridis* competitively eliminated *M. galloprovincialis*, however its presence and dominance of suitable substrate would make recolonization unlikely. The authors suggest the change may have been a result of warming sea conditions or pressure from native parasites, but causation is not clear and would require further study. Several native mussel species -in particular *M. galloprovincialis* - are present in the RAA and If such competitive exclusions were to occur within the RAA, commercially and ecologically important species may be adversely impacted. However, it is unclear whether the replacement of one species of reef forming bivalve with another would adversely impact wider communities. *P. viridis* is known to colonise seagrass beds in high densities (Baker et al., 2012, Rajagopal et al., 2006, S.t.A. Buddo et al., 2003). Impacts on seagrass health and biodiversity in seagrass beds appears not to have been studied.
2.14. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g.	N/A	N/A	*P. viridis* has not yet been recorded in the RAA.

decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?			
2.15. 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?	Major	Low	As conditions become increasingly favourable for *P. viridis* as a result of increasing SST in the RAA, the potential habitable and optimal range and reproductive success of the species will increase. This would likely result in an amplification of any of the potential impacts described in 2.13 and would increase the size of the area potentially impacted. *P. viridis* is known to colonise seagrass beds in high densities (Baker et al., 2012, Rajagopal et al., 2006, S.t.A. Buddo et al., 2003). *Posidonia* and *Zostera* beds are an important habitat in the Mediterranean and Atlantic respectively and are important nursery areas for fish an invertebrates as well as providing habitat for a diversity of other organisms. The impacts of *P.viridis* colonisation on seagrass health appear not to have been studied. However, in very high densities it could be predicted that smothering and available habitat for growth could occur. Biogenic reefs formed by bivalves, polychaetes worms and other species are also important for biodiversity within the RAA and potential impacts described in 2.13 would be likely to adversely affect the biodiversity value of these habitats. The potential to smother existing benthic communities described in 2.13 would additionally pose a threat to slower growing benthic organisms, including corals, sponges and large, solitary bivalves (e.g fan mussels) which could be smothered and overgrown. These species are found throughout the RAA.

2.16. 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?	N/A	N/A	*P. viridis* has not yet been recorded in the RAA.
2.17. 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?	Moderate	Medium	*P. viridis* is known to colonise seagrass beds in high densities (Baker et al., 2012, Rajagopal et al., 2006, S.t.A. Buddo et al., 2003). Although the impact of such colonisation is not currently known, given the high conservation importance of seagrass habitat within the RAA, this is an area requiring further study. Competition impacts of *P. viridis* on beds of other bivalve species have been reported in Florida (Baker et al. 2007). Reefs formed by bivalve molluscs (*Ostrea*, *Mytilus* and *Modiolus*) in the Mediterranean and Black Sea are listed under the EU Habitats Directive 'Reefs' designation as a priority habitat, and could be adversely affected by the arrival of *P. viridis*. As conditions become increasingly favourable for *P. viridis* as a result of increasing SST in the RAA, the potential habitable and optimal range and reproductive success of the species will increase. This would likely result in an amplification of any of the potential impacts described above and would increase the size of the area potentially impacted.
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	Moderate	Low	No specific information could be found to identify cultural ecosystem services in its non-native range. However, Mussels including *Perna sp* are known to consume plankton in high quantities (Zeldis et al., 2004). There is potential for this predation to impact species and habitats, which provide food, coastal defence and cultural services.

			When undertaking studies of *P. viridis* populations I New Mexico, Baker at al (2007) found that *P. viridis* growing over dead individuals on *Crassostrea virginica* reefs. In the USA, oysters are important commercially and culturally and also provide a coastal defence and water quality maintenance. Should *P. viridis* impair these reefs, such services may be adversely affected. *P. viridis* is known to colonise seagrass beds in high densities (Baker et al., 2012, Rajagopal et al., 2006, S.t.A. Buddo et al., 2003). Sea grass beds provide a range of services, including sediment stabilization, coastal defence and nursery area for commercially and culturally important species. The impact of such colonisation on these functions is not currently known, but it is likely that smothering would reduce the ability of beds to provide such services. Yamada et al. (2009) cite studies (in Japanese), which have shown a decline in *Mytilus galloprovincialis* , - which is native to the RAA but invasive in Japan - in Japanese waters. In some cases these seem to have been replaced by *P. viridis*. There is currently little evidence to suggest *P. viridis* competitively eliminated *M. galloprovincialis,* however its presence and dominance of suitable substrate would make recolonization unlikely. The authors suggest the change may have been a result of warming sea conditions or pressure from native parasites, but causation is not clear and would require further study. Additionally it is unclear whether the replacement of one species of reef forming bivalve with another would adversely impact the services provided.
2.19. How important is the impact of the organism on provisioning, regulating, and cultural services currently in	N/A	N/A	*P. viridis* has not yet been recorded in the RAA

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)?			
2.20. 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?	Moderate	Low	Mussels including *Perna sp* are known to consume plankton in high quantities (Zeldis et al., 2004). There is potential for this predation to impact species and habitats, which provide food, coastal defence and cultural services within the RAA. Specifically, reef-forming bivalve and worm species form biogenic reefs, which provide coastal defence, water quality maintainance and habitat for commercially and culturally important species (e.g. fish and invertebrates). Potential impacts on native species, some of which have cultural importance - for example competing with native mussels in the genus *Mytilus* might impact traditional, cultural activities. Many areas within the RAA have strong cultural ties to the harvesting and eating of shellfish, including mussels and oysters. These species are among those most likely to be negatively impacted by *P. viridis.* When undertaking studies of *P. viridis* populations in New Mexico, Baker at al (2007) found that *P. viridis* growing over dead individuals on *Crassostrea virginica* reefs. In the RAA, oysters, in particular *Magallana/ Crassostrea gigas* are important commercially and culturally (despite being an introduced – and in some cases invasive – species in the region itself). The cultural importance of theses oysters is particularly important on the southern coast of France, Spain and Portugal. *P. viridis* is known to colonise seagrass beds in high densities (Baker et al., 2012, Rajagopal et al., 2006, S.t.A. Buddo et al., 2003). *Posidonia* and *Zostera* beds

			are an important habitat forming species in the Mediterranean and Atlantic respectively and are important nursery areas for socially and commercially important fish and invertebrates. Beds provide other services in the RAA, including sediment trapping and coastal defence as well as a habitat for charismatic (and therefore culturally important) animals such as seahorses. The impact of such colonisation on these functions is not currently known, but it is likely that smothering would reduce the ability of beds to provide such services. Yamada et al. (2009) cite studies, which have shown a decline in *Mytilus galloprovincialis* , - which is native to the RAA and culturally and commercially important. In some cases invasive populations seem to have been replaced by *P. viridis*. It is unclear whether the replacement of one species of reef forming bivalve with another would adversely impact the services provided by the system.
Economic impacts			
2.21. 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	Major	Medium	Observed impacts include clogging of water pipes, leading to restricted flow, increased erosion and costly removal activity. This can impact power plants and other large-scale industrial operations using seawater for cooling (Rajagopal et al. 2006). A study in an Indian power station, in which *P. viridis* made up 87% of the fouling organisms, showed that continuous high-level chlorination was necessary to effectively control mussels in the cooling pipes, at high economic cost (Rajagopal et al. 1996). It is also considered likely that dense encrustations will impair the use of fishing/ mariculture gear; impair water flow in aquaculture operations leading to higher costs associated with commercial operations.

			Perna viridis also has an economic impact to shipping, as a frequent fouler of ships, navigation buoys and jetties (Baker et al. 2007, Rajagopal et al. 2006). Mussels including *Perna* sp are known to consume plankton in high quantities (Zeldis et al., 2004). There is potential for this predation to impact open water and coastal trophic interactions, in particular bivalve species of commercial importance might be negatively impacted through competition for food resources and predation of planktonic larva. Wells et al. (2017) report on the costs that authorities and industry have incurred during the process of detecting and intercepting incursions by *P. viridis* on vessel hulls. The authors note that costs are high, but efforts were focussed by utilising a risk assessment process to identify vessels to prioritise for inspection or cleaning. Whilst the inspections themselves resulted in 'significant cost' (no figure given) additional high costs were associated with subsequent management actions. These additional costs included delays to mobilisation and cost of cleaning. In the case of vessels too large to clean using facilities available, long additional journeys were required to travel overseas for cleaning. In the RAA, similar costs could be expected, but would vary depending on infrastructure already available and systems in place. It is worth mentioning also that such monitoring and control efforts would have the potential to target a range of fouling INNS, not just *P. viridis* and subsequently should not be considered as a cost associated with this species alone. When studying interactions between *P. viridis* and the oyster *Crassostrea virginica* in Florida, Baker et al. (2012) reported evidence that *C. virginica* had been overgrown and dominated by the mussel in some areas, especially subtidally. Oysters appeared restricted to

			intertidal areas. This is consistent with the findings of others that oysters have a far greater tolerance to extreme temperature fluctuations encountered in the intertidal than *P. viridis* (McFarland et al., 2014). These findings are of particular significance to operations in the Mediterranean, where tides are negligible and oysters are cultivated in areas likely to be colonised by *P. viridis* as any impacts could result in significant economic losses.
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	N/A	N/A	Not currently present in RAA, therefore not applicable.
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	Major	Low	Throughout the RAA, cultivation of the Pacific oyster *Magallana/ Crassostrea gigas* is commercially important. Mussel and oyster farming occurs throughout the Mediterranean and Black Seas' with a high economic value (e.g. 64,000 tonnes of mussels and 53 tonnes of Pacific oysters produced in Italy in 2013; FAO 2016). And although no interactions between this species and *P. viridis* have been reported, interactions with the closely related and ecologically similar *C. virginica* have identified potential impacts. Mussels including Perna sp are known to consume plankton in high quantities (Zeldis et al., 2004). There is potential for this predation to impact bivalve species of commercial importance might be negatively impacted through competition for food resources and predation of planktonic larva. This in turn might reduce quantity, condition and value of stock. Further observed impacts include clogging of water pipes, leading to restricted flow, increased erosion and costly removal activity. This can impact power plants and other large-scale industrial operations using seawater

			for cooling (Rajagopal et al. 2006). A study in an Indian power station, in which *P. viridis* made up 87% of the fouling organisms, showed that continuous high-level chlorination was necessary to effectively control mussels in the cooling pipes, at high economic cost (Rajagopal et al. 1996).
2.24. 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)?	N/A	N/A	Not currently present in RAA, therefore not applicable.
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	Major	Medium	If *P. viridis* becomes established in the RAA, eradication would likely not be an economically viable option to the open nature of the marine environment and life history traits of the species. Wells et al., 2017 estimate the cost of attempting to remove a fouling marine organism from the environment with a 5%-20 chance of success would cost an estimated AU$ 5-20 million. It is likely that measures would be required to prevent incursion into protected or commercially sensitive areas. Including vessel inspections and out-of-water cleaning. Depending on vessel size, this can be a considerable expense. It is likely that any costs associated with managing fouling of vessels and structures would not increase much beyond the current costs. Wells et al. (2014) point out that these costs would likely be offset by benefits to fuel efficiency and safety and the cost of removing *P. viridis* would not differ greatly to costs associated with removal of existing fouling communities. Removal from pipes and internal systems may be more costly due to the tolerance to chlorine (usually used in pipe cleaning) identified by Rajagopal et al. (1991).

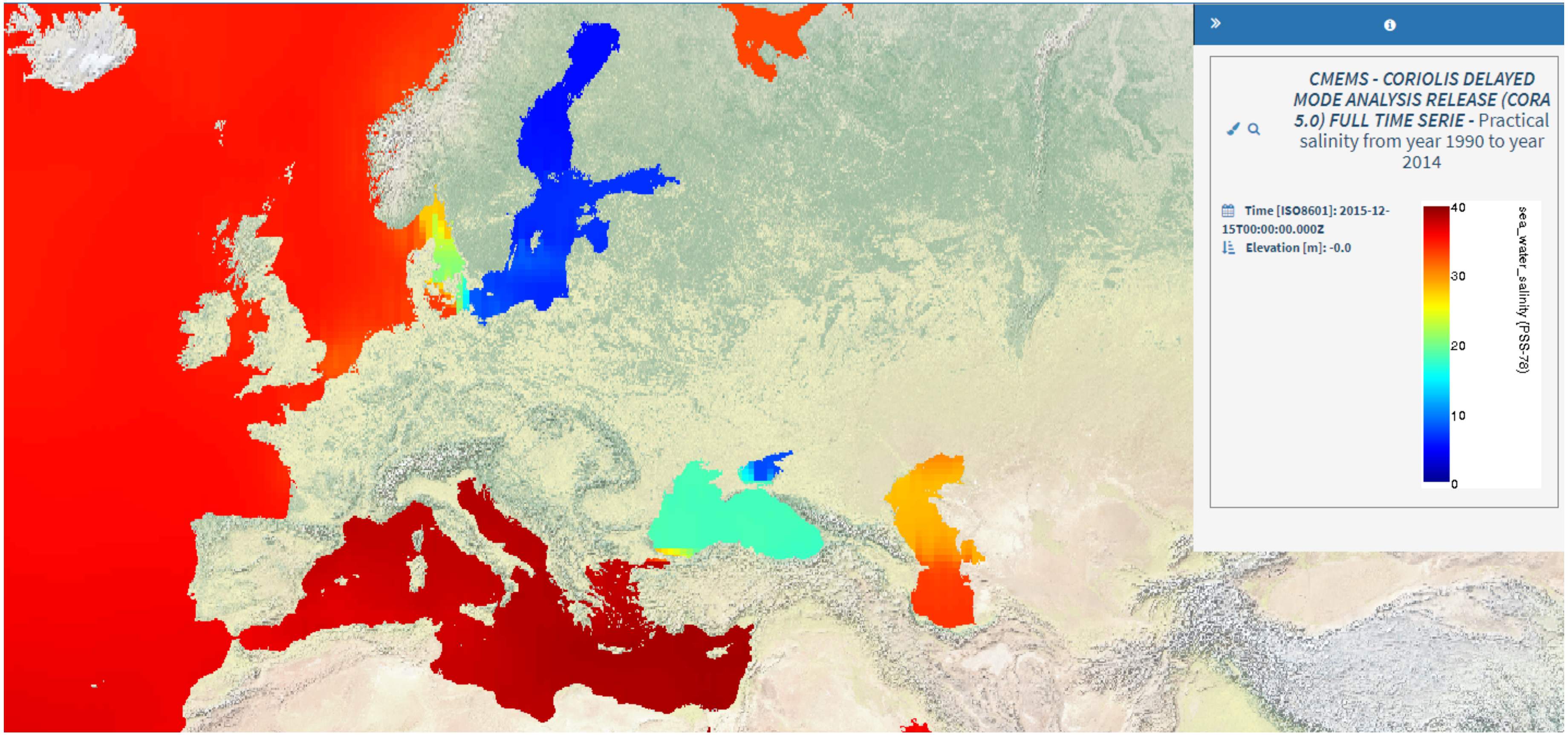
Social and human health impacts			
2.26. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).	Moderate	Medium	Fouling of recreational vessels and structures used for recreational activities is likely. Fouling of vessels can increase drag and consequently fuel consumption and cost. Clogging internal pipes and moving parts in industry can potentially impair safety (Rajagopal et al. 2006). However, this particular impact of *P. viridis* will add to an existing problem rather than creating a new one (Baker et al., 2007). Mussels including *P. viridis* accumulate high levels of metals and contaminants from their environment and if consumed, these may have adverse impacts on human health. Hg levels can in some cases pose a threat to human health even if consumed at low levels (Fung et al., 2004). The level of threat is however related to local water quality and would be an issue in other (native and alien) mussel species also. Buddo et al. (2012) describe the ability of *P. viridis* in Jamaica to accumulate a range of toxins and biological pathogens, which could easily be transferred to humans if eaten. Illegal and/ or unrestricted harvesting and consumption is likely I the risk assessment in particular in areas where recreational shellfish gathering is common. This would increase the likelihood of humans consuming infected mussels and could lead to serious health issues. This species has been particularly implicated in accumulation of toxins associated with Paralytic Shellfish Poisoning (Yen et al. 2004, 2006). This threat to human health would be directly related to existing water quality within the area of establishment and it is anticipated that regulations governing the extraction of shellfish for food would limit the realised impact on human health.

2.27. 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.	Moderate	Low	As conditions become increasingly favourable for *P. viridis* as a result of increasing SST in the RAA, the potential habitable and optimal range and reproductive success of the species will increase. This would likely result in an amplification of any of the potential impacts described in 2.26 and would increase the size of the area potentially impacted.
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	Major	Medium	*Perna viridis*, like all water-filtering bivalves, has the potential to accumulate 'red tide' toxins from phytoplankton, which pose a risk for human consumption (Buddo et al. 2003; Rajogopal et al. 2006). Paralytic Shellfish Poisoning associated with this species has been reported in the Caribbean (Yen et al. 2004, 2006). Protozoan parasites are known from this species (Tuntiwaranuruk et al. 2004), and *Cryptosporidium sp.*, which may pose an infection risk to humans if eaten, has been found in *P. viridis* in Thailand (Srisuphanunt et al. 2009), but the risk of these parasites if transported to the RA area is unknown.
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	NA	N/A	No additional impacts currently known
2.30. 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?	Moderate	Low	*P. viridis* is a highly fecund, fast growing species, tolerant of a wide range of environmental conditions. It is therefore capable of recovering from pressures exerted by other species. In the RAA, a number of potential predators exist, these include: Starfish, predatory gastropods, fish, seabirds, crabs and lobsters.

			These species have adapted to feed on mytilids and other native mussel species and it is likely that they would also be able to take *P. viridis* as a prey species. Globally, bed-forming mussel species are known to undergo mass mortalities due to predator pressure, in particular from starfish, which may occur sporadically in large numbers, decimating mussel beds. Such events might impair the ability of introduced *P. viridis* to thrive and could potentially reduce impacts. Many similar predators exist within the natural and introduced range and given the right conditions, it has become established. *P. viridis* utilises number of anti-predator behavioural traits including increased byssal thread production and clumping behaviour (Cheung et al. 2004, Wang et al. 2013). Although not certain, It has been suggested that one reason why *P. viridis* has failed to become established in Australia despite thousands (Heersink et al., 2014, Wells 2017) of introductions is the presence of a large number of potential predatory species. Predation combined with sub-optimal conditions may reduce the ability to become established. *P. viridis* is able to adapt its shell morphology due to the presence of predatory species, potentially resulting in reduced predation (Cheung et al. 2004). Pea crabs have been found within *P. viridis* during studies in Malaysia (Al-Barwani et al., 2011) and have been found to inhabit and impair the condition of *P. viridis* in Japan (Yamada et al., 2009) and India (Jose & Deepthi 2005). These commensal parasites are known to reduce the condition of mussels and reduce reproductive potential. However, to what degree European pea-crab species will inhibit establishment is unknown.

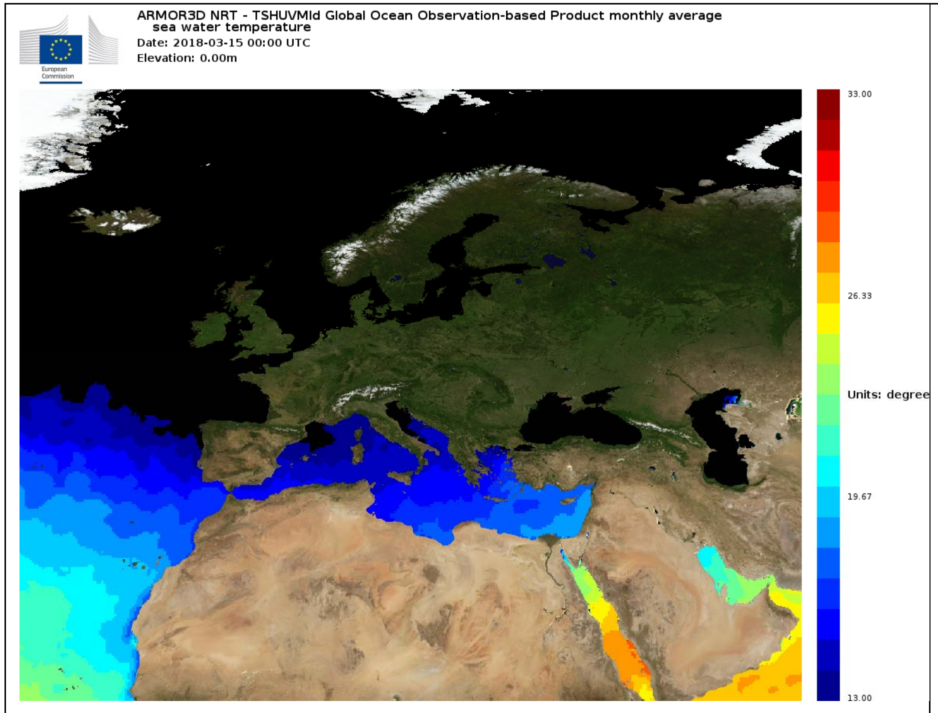
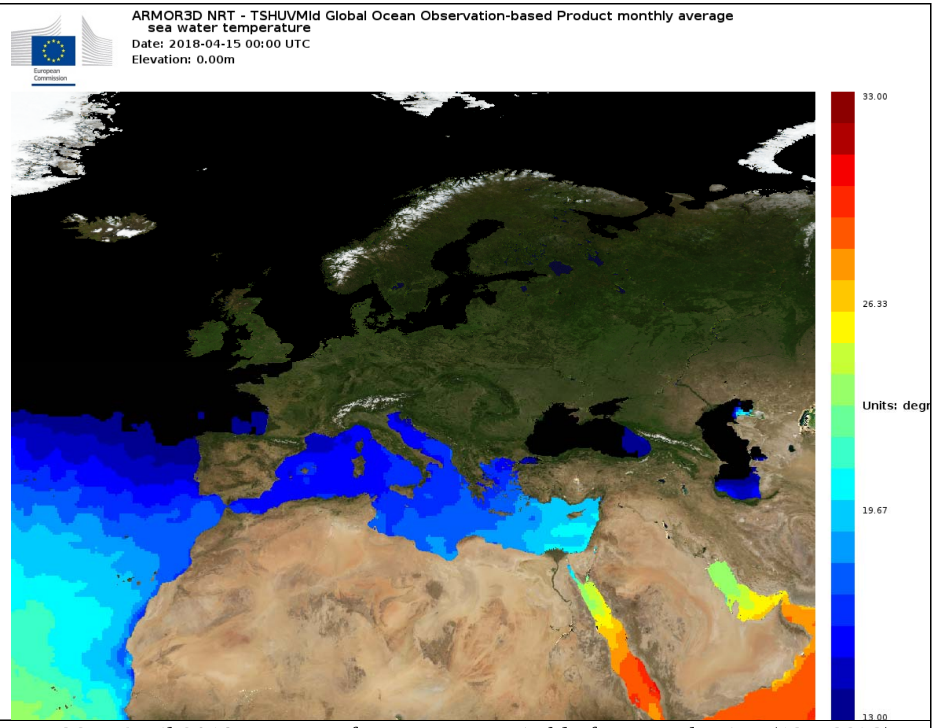
			Mass mortality events have been observed in *P. viridis* as a result of blooms of the toxic dinoflagelate *Karenia brevis* (McFarland et al., 2015, Baker et al., 2012). This species has been recorded in the Mediterranean and North East Atlantic alongside its congener *K.mikimotoi*. However mass mortalities were not observed during blooms of *Pyrodinium bahamense* which produces saxotoxin (Baker et al., 2012). Skein et al. (2018) studied the interactions between two predators of bivalves with two introduced mussel species and found that the predatory starfish and lobster fed preferentially on native species of mussel, preferring them to the non-native mussel species. Such experiments highlight the potential for introduced species to escape predation by unfamiliar predatory species. To the best of the authors' knowledge there has been no detailed assessment of the disease profile of this species within its native or introduced range. It is therefore impossible to make comment on what diseases movement of this species may carry and how these may have further impact on the ecosystem it is introduced into. The lack of disease profiling of invasive/horizon species has been highlighted on multiple occasions (e.g. Roy et al. 2017).

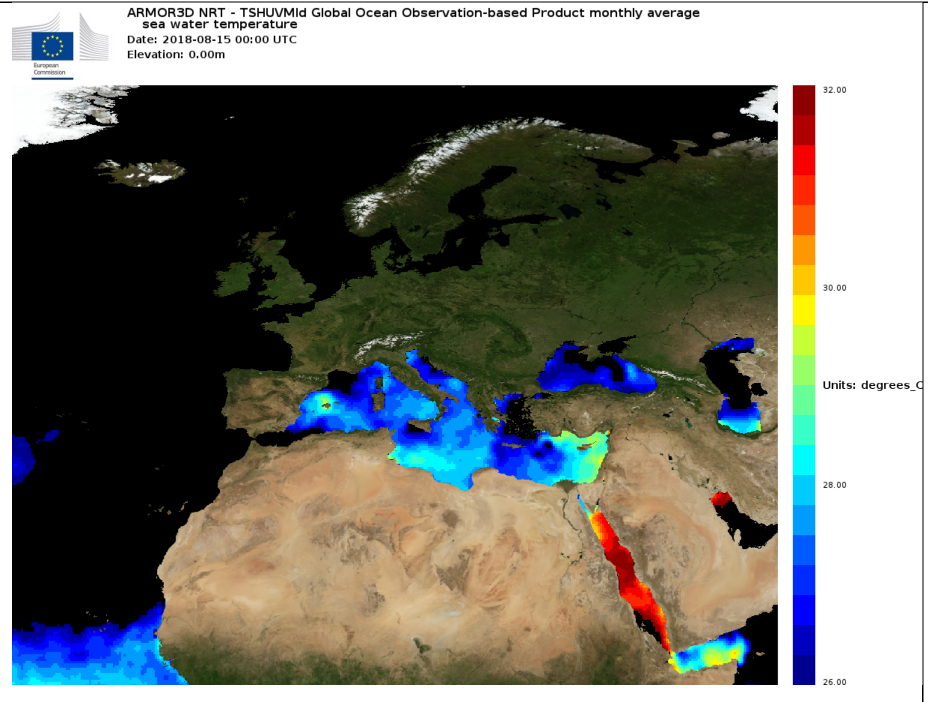
Appendix: Climatic variables maps



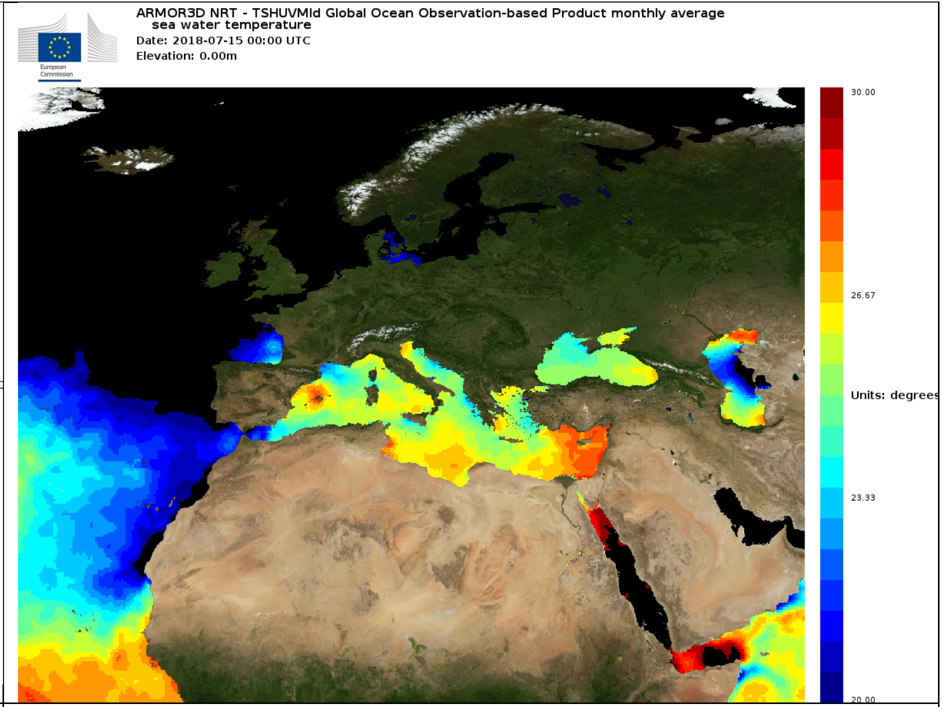
Mode analysis of salinity in European sea regions 1990 – 2014. Data used as reference for approximate salinity conditions in the RAA. Data from: http://www.emodnet-physics.eu/Map/Products/V2/PRODUCTS.aspx?PRODTYPE=CL&type=PSAL&param=salinity&CLtype=CMEMS
Future Predicted changes: Baltic likely to reduce by 50-80% due to ice melt (EEA 2017)

Copernicus Global ARMOR3D L4 product Global Ocean Observation Based Mean Monthly SST and Salinity Data From: https://goo.gl/yJPw29

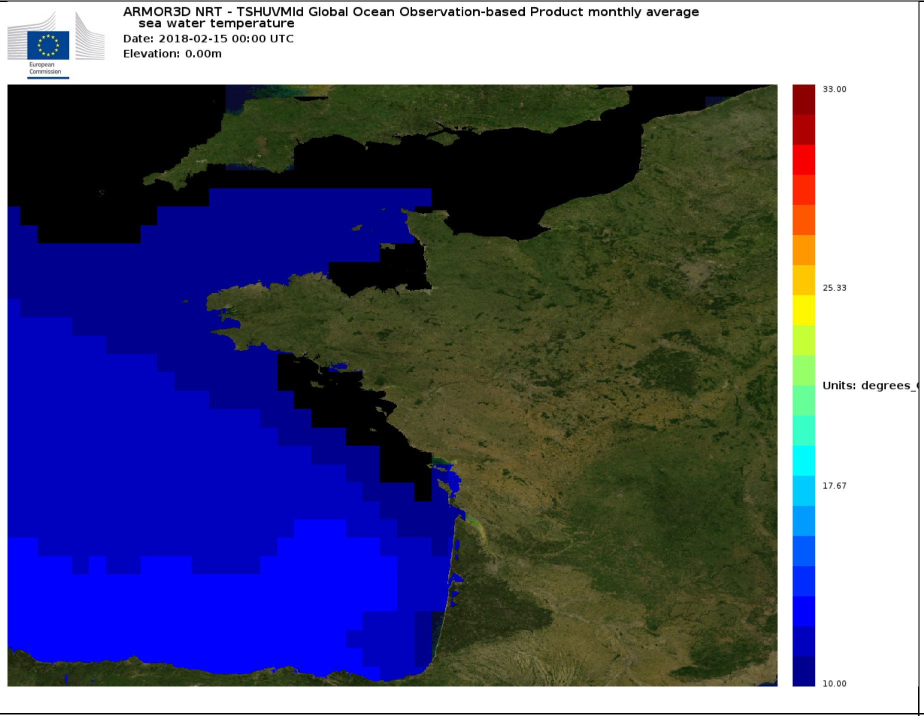
	
MMSST: March 2018 – Range of temperature suitable for reproduction (13 – 33ºC) – Black area is considered outside range of temperature under which reproduction is known to occur.	MMSST: April 2018 – Range of temperature suitable for reproduction (13 – 33ºC) – Black area is considered outside range of temperature under which reproduction is known to occur.



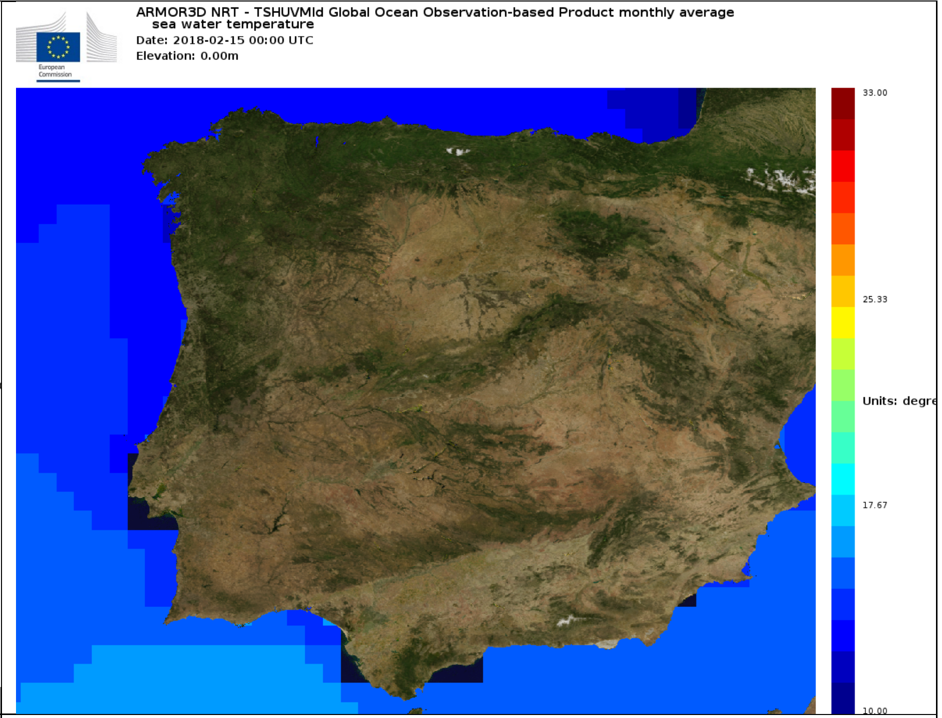
MMSST: August 2018 – Optimum Temperature for reproduction (26 – 32ºC) – Black areas are considered outside optimal range.



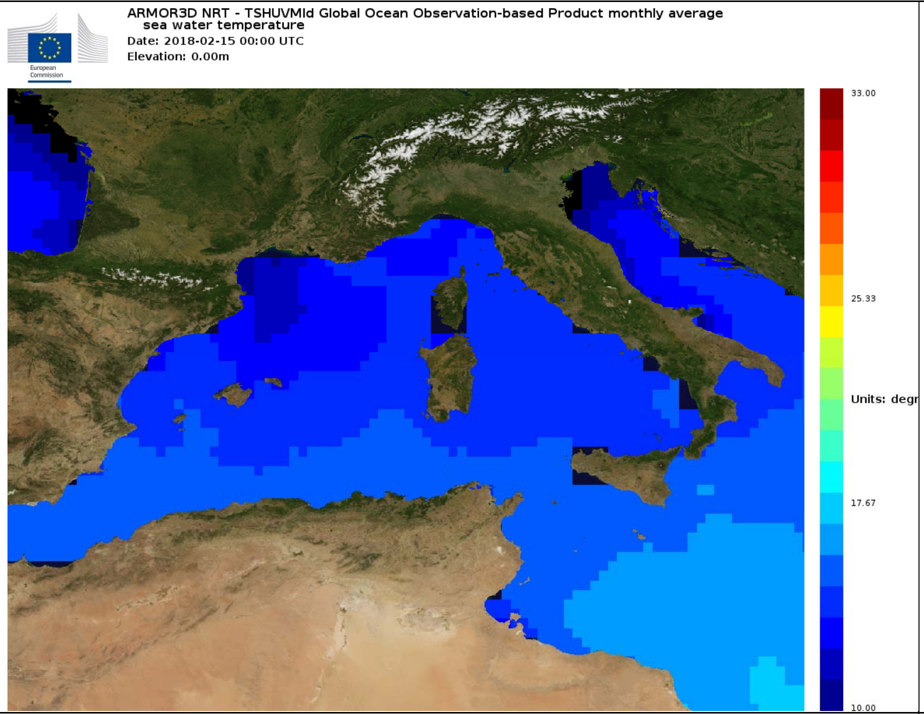
MMSST: August 2018 (20 – 30ºC) – Also within higher tolerable temperature range.



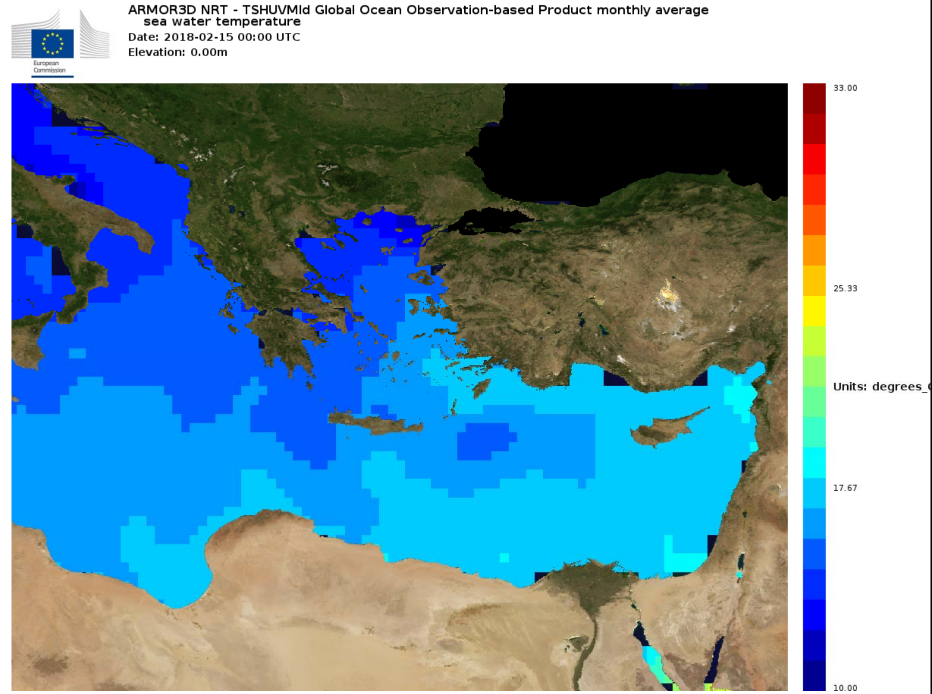
MMSST: Feb 2018 – Min Survivable Temperature (10 – 33ºC) Bay of Biscay – Black area is considered too cold for winter survival.



MMSST: Feb 2018 – Min Survivable Temperature (10 – 33ºC) Spain Portugal – Black area is considered too cold for winter survival.



MMSST: Feb 2018 – Min Survivable Temperature (10 – 33ºC) Central Med' – Black area is considered too cold for winter survival.



MMSST: Feb 2018 – Min Survivable Temperature (10 – 33ºC) Easter Med – Black Sea – Black area is considered too cold for winter survival.

ANNEX I	Scoring of Likelihoods of Events
ANNEX II	Scoring of Magnitude of Impacts
ANNEX III	Scoring of Confidence Levels
ANNEX IV	Ecosystem services classification (CICES V5.1) and examples
ANNEX V	Biogeographic Regions and MSFD Subregions

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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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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 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.

[10] Not to be confused with „no impact".

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.*. 2017)

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[11]	**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.*

[11] 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.

	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** 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

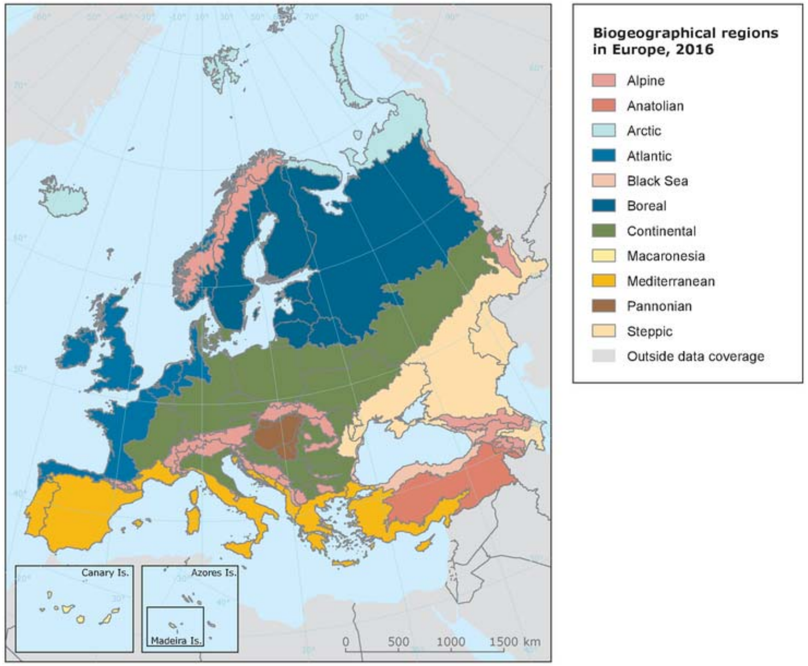
			Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific 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.*

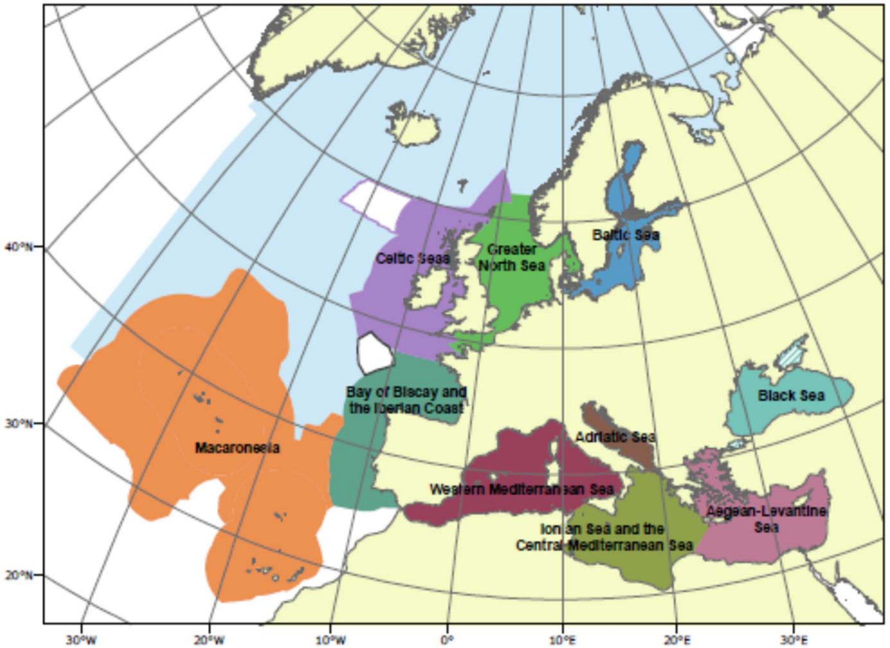
ANNEX V EU Biogeographic Regions and MSFD Subregions

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 with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	*Perna viridis* (Linnaeus, 1758)
Species (common name)	En) Green-lipped mussel; (En) Asian green mussel; (En) green mussel (fr) moule verte asiatique; (NL) Aziatische groene mossel; (De) Asiatische grüne Miesmuschel
Author(s)	Jack Sewell, The Marine Biological Association, UK Paul Stebbing, Cefas, UK Phil Davison, Cefas, UK
Date Completed	19/09/18
Reviewer	Marika Galanidi, Dr, University, Institute of Marine Sciences and Technology, Izmir, Turkey Argyro Zenetos, Dr, Hellenic Centre for Marine Research, Greece

Summary

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.

Prevention of introduction:

Ballast water movements: The Ballast Water Management Convention entered into force on the 8th September 2017 and is currently in the process of being implemented. Although it is an existing practice, its current effectiveness of limiting introductions from occurring will be comparatively low in relation to what it will be once implementation has occurred. By the end of the lead in time for the Convention all countries will need to be applying the convention within scope of the articles of the Convention. Until the Convention has been fully implemented then the risk of the organism surviving passage via this pathway remains High.

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 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. Therefore, the organism is considered likely to be able to survive passage.

Deliberate introduction for aquaculture: Current legislation in the form of the Alien Species in Aquaculture Regulations (708/2007) prevents the deliberate introduction of non-native species for aquaculture, unless potential risks posed by the species are mitigated within EU member states. To establish an aquaculture site within the EU for this species the organism would need to be risk assessed under the regulations. This is likely to highlight that the species cannot be farmed without extensive mitigating measures (e.g. only farmed in enclosed indoor recirculating systems) put in place making any venture more expensive and therefore less desirable, or altogether impossible. In order for this legislation to remain effective at preventing arrival through intentional introduction, continued effective enforcement across the risk assessment area is required.

Early detection:

National monitoring programmes: Under the Marine Strategy Framework Directive (2008/56/EC) each Member State is required to implement a monitoring programme to assess the status of the 11 descriptors included in the directive, the second of which (descriptor 2) is concerning non-native species. In supporting documentation Commission Decision 2017/848 one of the (three) criteria for assessing descriptor 2 is the rate of introduction of new non-native species. Therefore, any supporting monitoring programme needs to be able to detect new introductions. This will in turn facilitate national rapid response processes. Monitoring programmes under the MSFD are starting to become more developed and robust in detection of marine non-native species, but there is still considerable work that need to be done in some cases to fully support rapid response processes.

e-DNA detection: New monitoring methods are constantly being developed to improve efficiency or detection rates (Bean et al. 2017). A method that has potentially a lot of application for detecting marine non-native species is the use of environmental DNA (eDNA). Although still being developed for full scale effective deployment in the marine environment, eDNA may well aid in the rapid and early detection of no-native species.

Rapid response:

Processes: Several countries within the Risk Assessment Area have rapid response processes established. These processes would be instigated in the case of the detection of a species as invasive as *P. viridis.* Methods have been developed elsewhere, for example in Australia, and these methods would be used to control the species if it was introduced elsewhere (Heersink et al 2014). This does however rely on the introduction being detected early in the invasion process and sufficient resources being made available to implement the controls. Given morphological similarity to native mussel species found in the area, monitoring and interception would require fairly specialist training and identification resources and trained experts to maintain regular monitoring in sites where new introductions would be most likely to occur.

Eradication methods: If a decision is made to attempt an eradication upon detection of the species there are several actions that could be undertaken, mainly based on where the population has been found, resources available and the potential for collateral damage as a result of application. Methods of treatment identified by the Australian authorities for managing the species includes i) draining, ii) flushing with freshwater and/or hot water, iii) chemical biocides, and iv) physical removal.

Prevention of spread and management:

A decision may be made not to try and eradicate, but to implement a containment and control process. This may include movement controls of potential vectors in addition to processes to limit the species distribution and population size to reduce impact and propagule pressure (i.e. limiting the risk of spread). The options for vector control will include ballast water and hull-fouling, as above, but will also need to be assessed on case by case basis depending on the use of the site. For example, marinas may want to put addition controls in place relating to recreational equipment, or an aquaculture site will need to consider methods of checking stock for contamination. Basic principles for (certain) vector control and species management include i) draining, ii) flushing with freshwater and/or hot water, iii) chemical biocides and iv) physical removal.

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods of managing pathways (including prevention of introduction and spread)	Ballast Water Control: Effective control of ballast water exchange to reduce potential for introduction of *P.viridis* propagules into risk assessment area.	The Ballast Water Management Convention is complex and requires the establishment and running of infrastructure. Initially the Convention will need to be translated into a domestic statutory instrument by which enforcement activities can be instigated. How and to what extent the Convention is conveyed will determine the potential costs and cost effectiveness. In addition to enforcement (including suitable testing of samples as part of the process) of the Convention costs will include monitoring to determine effectiveness and the establishment of warning systems (to communicate to operators where ballast water should not be taken up due to the present of harmful algal blooms, presence of sewage outfalls or invasive species), in addition to setting up intrastate such as on land sediment reception facilities. In relation to monitoring, certain elements of this maybe covered by the MSFD monitoring requirements. There are also some additional options that Member States may wish to adopt, for example, offering operators the ability to be given exemption	High

		for vessels travelling on dedicated routes. These exemptions will be based on risk assessments processes, which will be paid for by the operator and valid for a maximum of 5 years. It is therefore very difficult to attribute a cost to Member States in relation to the costs of implementation. The state incurred implementation costs is likely to be in access of $1M, for example Croatia estimated a cost of $1.4M (Interwies & Khuchua 2017), while the running costs are likely to be >$100,00 per annum (as estimated for the Bahamas), however, these costs are very approximate estimates and will vary considerably between Member States, depending on extent of implementation, national policy and number of ports. The Convention introduces standards by which ships are required to operate, D1- ballast water exchange, where 95% ballast exchange is required to be conducted at least 200 nautical miles from the nearest land and in water at least 200 metres depth, and D2- ballast water management, where the vessel is fitted with a type approved treatment system. D1 is only a temporary standard whereby vessels are supposed to transit to a point where they have a ballast treatment system fitted. While D1 is relatively in expensive for operators, the installation costs of ballast water management systems can be considerable – estimates by ship-owner organisation BIMCO suggest up to $5 million (USD) per ship – and operational costs of the systems over the ship's lifetime could be even higher (www.ballastwatermanagement.co.uk). For example, Colombia estimated an implementation cost of $81million based on 52 vessels as a basis for their calculations operating under their flag (Interwies & Khuchua 2017). Ballast water exchange has been proven to be very effective at reducing the risk from the introduction of non-native species via this vector. For example, the Great Lakes in North America have seen a considerable decrease in the number of species	

		introduced by ballast since the introduction of ballast water management in 2006. There does appear to be some questions over the effectiveness of ballast water treatment systems, for example how effective they are under different salinity and temperature regimes.	
	Hull-fouling control: Treatment of vessel hulls with substances which make settlement and attachment of *P.viridis* difficult, resulting in a reduction of risk of transfer by this vector.	Hull fouling is controlled via anti-fouling paints and cleaning practices both in the commercial and recreational sectors. Paints however have limited service life and require re-application at regular intervals (Rajagopal et al 2006). The majority of hull fouling paints are copper based. *P.viridis* has been shown to be susceptible to copper toxicity (Chan 1988). If anti-fouling paints are not maintained, then the risk of transfer by this vector is increased. 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. 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. Alcohol extracts from gorgonian corals have shown some promise as a potential antifouling substance, inhibiting growth of bysal threads in *P.viridis* and inhibiting settlement (Wilsand et al 1999). However *P.viridis* is far more tolerant of naturally occurring antifoulants than other fouling species, suggesting use of these substances may provide a competitive advantage to *P.viridis* if used.	High

		The physical removal of hull-fouling has been suggested as a means of control invasive species (Frey et al 2014). In water cleaning runs the risk of leading to the release or dispersal of viable non-native species facilitating introduction, while also potentially damaging anti-fouling paints limiting effectiveness. No specific costs could be found for the application of anti-fouling systems within the UK, but as anti-fouling is a common practice, then technically speaking no additional costs would be incurred in relation to this species.	
	Deliberate introductions control: Prevention of intentional introduction into the wild for commercial harvesting.	Current EU legislation in the form of the Alien Species in Aquaculture Regulations (708/2007) prevents the deliberate introduction of non-native species for aquaculture, unless potential risks are mitigated within Member States. To establish an aquaculture site within the EU for *P. viridis* the organism would need to be risk assessed under 708/2007. While the legislation presents a robust road-block to the potential introduction of the species, any legislation is only as good as its enforcement. Maintaining high levels of enforcement in relation to these regulations is therefore essential. As this legislation is already implemented there would be no additional costs associated specifically with this species.	High
Early detection and rapid response	Early detection: national monitoring programmes and novel detection methods to facilitate rapid response processes.	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	High

		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 *P.viridis* (see Micklem et al 2016) - in particular similarities to native 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.	
	Rapid response processes: early response to newly detected introductions.	The European Union Regulation (No 1143/2014) on the prevention and management of the introduction and spread of invasive alien species (IAS) entered into force in 2015. A key instrument of the Regulation is the List of IAS of Union concern, known as 'the Union list'. Species that are included in this list	High

		are subject to a number of measures including prevention, early detection and rapid eradication of new invasions. An Early Warning and Rapid Response System (EWRRS) has been designed by the European Alien Species Information Network (EASIN) and is now under development, to support the new European Regulation on the prevention and management of the introduction and spread of invasive alien species (IAS). Although this process very much focuses on listed species, non-listed species such as *P. viridis* would still fall under the requirements of Member States to respond to new introductions. Rapid responses process would need to be established specifically for this species (or similar species). As this would involve potentially maintaining capacity to respond to new introductions it is very difficult to determine the costs associated with this single species alone. Understanding the points of introduction can help to focus resources for both monitoring and rapid response. Processes have been developed to assess hot spots of introduction (Tidbury et al 2016) and establishment (Heersink et al 2004). The application of these methods would greatly enhance predictive capabilities of responses.	
Eradication and control methods.	draining/air exposure	*P.viridis* is intolerant to desiccation (McFarland et al 2014). Draining down of suitable locations, could aid in controlling the species, removing it from certain locations, such as power plants, raw water systems, reservoirs, locked marinas and impoundments. Likewise, the drying of bio-fouled equipment (e.g. pontoons), would also aid in removing the species from specific locations. Similar processes could be used for smaller equipment, which may facilitate the transfer of the organism. This process may prove an effective and low-cost method for reducing the potential of spread by equipment used in marine	High

		based activities. Specific costs will vary depending on the type of equipment and the demand on it use (to allow time for it to dry).	
	flushing with freshwater and/or hot water	*P. viridis* can survive prolonged exposure to freshwater/decreases in salinity (Rajagopal et al 2006). The application of this method would also be limited to enclosed systems where incursion from sea water can be prevented. To achieve eradication using this method the infested area would need to be exposed to freshwater water for a month or more. This process, due to the time of exposure required would not be a suitable bio-security measure. While this could present a cost effective and easily applied control method this is circumstantial. The Australian governments Marine Pest Plan presents evidence demonstrating that *P. viridis* is resilient to increased water temperatures, with 100% mortality occurring after 30 minutes exposure at 60°C. Although difficult to apply to large items of equipment or whole water systems, this method could be applied to smaller equipment as a bio-security measure, if the item being treated is not damaged by the treatment process. Estimating costs for these treatments, given their circumstantial application is impossible.	
	Chemical treatments	Chlorination of cooling pipes is a widely used method for controlling fouling. *P.viridis* can be controlled using this method, however Concentrations and duration of chlorine introduction would likely be higher than required for other species and might represent additional cost. study in an Indian power station, in which *P. viridis* made up 87% of the fouling organisms, showed that continuous high-level chlorination	High

		was necessary to effectively control mussels in the cooling pipes, at high economic cost (Rajagopal et al. 1996). *P.viridis* is more tolerant of higher chlorination levels than many fouling species found in the area (Rajangopal et al 2006), however treatment at high levels over prolonged period may be effective. Low concentrations of chlorinated water released continuously from power station has been used as a means of preventing settlement as it deters settlement (Rajangopal et al 2006). The use of chlorine also comes with considerable health risks to the user, this limits greatly the application of this method. Treatment with chemicals may prove effective and cost-effective method of managing populations in confined spaces (e.g. in power station cooling pipes) however in open water conditions, efficacy of chemical treatment may be limited.	
	Physical Removal	In 2001 individuals were intercepted in Cairns, Australia and removed by hand. Following this initial removal, an intensive eradication and monitoring process identified and destroyed a number of established individuals nearby (Heersink et al 2014, Baker et al 2007). Since this time, whilst a number of other introductions have occurred, interception and removal are likely one reason that *P.viridis* has not yet become established in the region. Such monitoring, interception and removal programmes are likely to be costly and would need to be ongoing. In the marine environment such methods may not be able to provide 100% coverage and effectiveness. However, evidence from Australia suggests that if carefully undertaken and effectively targeted, it might be an effective method. Mechanical and indiscriminate removal using dredge and other techniques used in commercial shellfish harvesting may prove more cost	Medium

		effective than targeted removal, however impacts on natural habitat and species may be significant and long lasting. Methods deployed to harvest and relay mussel seed from the wild might reduce numbers but given the heterogonous nature of the seabed in the area at risk of invasion, is unlikely to remove 100% of mussels and as such, would not be likely to eradicate the species effectively. Although interception and removal has been suggested as an eradication method, once established, eradication would be almost impossible. This approach would therefore be more appropriate as a population management method. Similar interceptions have been effective in Australia (Baker et al 2007), however, it is not clear how other variables (predation, environmental conditions etc) might have influenced the ability of *P.viridis* to become established. The authors were unable to find examples of similar schemes successfully eradicating or even controlling invasive fouling organisms similar to *P.viridis* in the marine environment in the risk assessment area or similar environments. Although physical removal programmes are unlikely to result in eradication, unless targeting newly established populations, and are likely to be costly; their potential application as a control method should be considered.	

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Name of organism: *Lagocephalus sceleratus* (Gmelin, 1789)

***Photo credit:* G. Kondylatos (HCMR)**

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[1] This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

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Risk Assessment Area: European Seas (excluding the outermost regions)

Peer review 1: Jack Sewell, The Marine Biological Association, Plymouth, United Kingdom
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Peer review 3: Wolfgang Rabitsch Environment Agency Austria, Department of Biodiversity and Nature Conservation, Vienna, Austria

This risk assessment has been peer-reviewed by two independent experts and discussed during a joint expert workshop. Details on the review and how comments were addressed are available in the final report of the study.

Date of completion: 25.10.2018

Contents

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**[2]	**COMMENT**
Summarise Entry[3]	very likely	high	*L. sceleratus* entered the Mediterranean Sea through the Suez Canal, a pathway that is still active and will likely provide opportunities for additional introductions. The species is already established in the RA area through natural dispersal from the recipient neighbouring countries and is continuously expanding its range throughout the Mediterranean. Further unaided introductions are expected in currently uninvaded regions. The likelihood of new introduction events through escape from public aquaria is considered to be low as the species is currently only displayed in public aquaria located in areas where establishment has already occurred.
Summarise Establishment[4]	very likely	high	Based on eco-physiological requirements and the distribution modelling, *L. sceleratus* is considered likely to establish further populations in the Western Mediterranean, with higher probabilities of establishment along the western Italian coast, Sardinia, Corsica and the eastern part of Mediterranean France, and a lower probability of establishment in the Gulf of Lyon and the Spanish Mediterranean coast, where temperatures close to the thermal limits of the species offer less favourable conditions both for the survival of the young fish and for prolific spawning. In the Atlantic *L. sceleratus* is moderately likely to establish only as far north as southern Spain and southern Portugal. By the 2050s, under the both the moderate RCP4.5 and the

[2] In a scale of low / medium / high, see Annex III

[3] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

[4] In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

			extreme RCP8.5 climate change scenarios, suitability in the Mediterranean increases, but the model predicts little potential expansion of the suitable region into the Atlantic.
Summarise Spread[5]	rapidly	high	*L. sceleratus* is a large fish that can reach up to 110cm length and 7kg, has high fecundity, extended pelagic duration (larvae and juveniles), few predators and competitors and is showing signs of adaptability to temperature conditions cooler than those in its native range. All these characteristics, along with favourable conditions have contributed to the rapid spread of the species throughout most of the Mediterranean Sea. Further spread is expected into currently uninvaded areas as described in the Risk of Establishment summary and population explosions may be triggered by one particularly warm summer.
Summarise Impact[6]	major (environment) massive (socio-economy)	medium low	In the East Mediterranean, both inside and outside the RA area, *L. sceleratus* has already attained significant densities and constitutes a worrying proportion of the ichthyofauna biomass in shallow waters, based on artisanal and small-scale fisheries catches. It is a voracious predator, with an ontogenetic shift in its diet from crustaceans and small fish to predominantly cephalopod molluscs as the age increases. It is notorious for attacking fish caught in fishers' nets and destroying the catch. Declines in wild stocks and catches of cephalopods, crustaceans and commercial fish species (e.g. red mullets) at the local scale have been attributed to predation by *L. sceleratus* and may be anticipated to extend to a larger area, however, due to the poor level of documentation of the existing impacts, high uncertainty is associated with this assessment. The

[5] In a scale of very slowly / slowly / moderately / rapidly / very rapidly

[6] In a scale of minimal / minor / moderate / major / massive, see Annex II

			preference of spawning aggregations for *Posidonia oceanica* meadows raises concerns for the conservation value of these habitats that act as nursery and feeding grounds for many fish species in the Mediterranean. Currently, the most severe impacts of *L. sceleratus* are the socio-economic ones. The species causes extensive damages to the gear and the catch of small-scale fishermen, causing major economic losses (that can potentially become massive in the future) that have already led some fishermen to abandon fishing as a livelihood activity. Finally, the consumption of this highly toxic species has led to numerous severe poisoning incidents and fatalities and, despite fishing and marketing bans on the species and numerous awareness campaigns, unsuspecting consumers still remain vulnerable. The number of the people at risk is expected to increase with further establishment of *L. sceleratus*.
Conclusion of the risk assessment[7]	high	medium	The organism is already present in the RA area, with well-established populations that are continuously spreading and steadily increasing to devastating densities at some locations. Despite the lack of strong evidence, there are indications for potentially major environmental impacts through predation, while the demonstrated socio-economic impacts are severe both in terms of economic losses and with regards to human health risk. *L. sceleratus* is therefore considered a high risk invasive species for the EU.

[7] In a scale of low / moderate / high

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A.
The answers in the tables below indicate the following:

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

Member States

Member States	Recorded	Established (currently)	Established (future)	Invasive (currently)
Austria				
Belgium				
Bulgaria				
Croatia	Yes	Yes	Yes	-
Cyprus	Yes	Yes	Yes	Yes
Czech Republic				
Denmark				
Estonia				
Finland				
France	-	-	Yes	-
Germany				
Greece	Yes	Yes	Yes	Yes
Hungary				
Ireland				
Italy	Yes	Yes	Yes	??
Latvia				
Lithuania				
Luxembourg				
Malta	Yes	No	Yes	??
Netherlands				
Poland				
Portugal	-	-	Yes	-
Romania				

Slovakia				
Slovenia	-	-	Yes	??
Spain	Yes	-	Yes	-
Sweden				
United Kingdom				

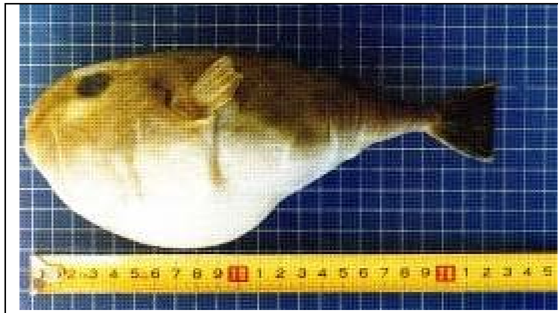
Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Baltic Sea				
Black Sea	Yes	-	Yes	-
North-east Atlantic Ocean				
Bay of Biscay and the Iberian Coast	-	-	Yes	-
Celtic Sea				
Greater North Sea				
Mediterranean Sea				
Adriatic Sea	Yes	Yes	Yes	??
Aegean-Levantine Sea	Yes	Yes	Yes	Yes
Ionian Sea and the Central Mediterranean Sea	Yes	Yes	Yes	Yes
Western Mediterranean Sea	Yes	Yes	Yes	Yes

SECTION A – Organism Information and Screening	
Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Phylum: Chordata Class: Actinopterygii Order: Tetraodontiformes Family: Tetraodontidae Species: ***Lagocephalus sceleratus* (Gmelin, 1789)** Synonyms: *Fugu sceleratus* (Gmelin, 1789) *Gastrophysus sceleratus* (Gmelin, 1789) *Pleuranacanthus sceleratus* (Gmelin, 1789) *Sphoeroides sceleratus* (Gmelin, 1789) *Tetraodon bicolor* Brevoort, 1856 *Tetraodon blochii* Castelnau, 1861 *Tetraodon sceleratus* Gmelin, 1789 names used in commerce (if any) global: Fugu, Silver-cheeked toadfish, Ballon à bande argentée Australia: Giant toadfish, Silver toadfish Malaysia: Silverstripe blaasop, Buntal

	Philippines: Spotted rough-backed blowfish, Tinga-tinga Turkey: Balon balığı No subspecies, varieties, breeds or hybrids are known.
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 wild, in confinement or associated with a pathway of introduction]	The family Tetraodontidae has representatives of many similar alien fish in the Mediterranean Sea namely *Lagocephalus guentheri* (Richardson, 1844); *Lagocephalus suezensis* Clark and Gohar; *Torquigener flavimaculosus* Hardy and Randall, 1983; *Sphoeroides pachygaster* (Müller and Troschel, 1848) but only one native species, the oceanic puffer fish *Lagocephalus lagocephalus* (Linnaeus, 1758). *L. lagocephalus* is quite distinct and readily distinguishable from all the members of the Tetraodontoidea by: (1) The presence of spines on the ventral side only; (2) The simple nostrils; (3) The large size (60 cm); and (4) The four rooted spines (Andrews, 1970). *Lagocephalus sceleratus* has an elongated, somewhat laterally compressed and inflatable body. No scales on the body, except for small spinules on the belly and on the dorsal surface extending to origin of dorsal fin. Dorsal and anal fins located far posteriorly, containing no spiny rays. *L. sceleratus* is readily distinguishable from all the members of the Tetraodontoidea by its colour [back and upper flank silvery to grey covered with black dots. Bright silver stripe on the side; belly white] and size (20-60 cm (max. 85 cm) (Golani et al., 2002). Its con-generic *Lagocephalus guentheri* bears small spinules on the dorsal surface, not extending posteriorly beyond the pectoral fin margin. In addition, its colour is back-dark grey (adults) to olive-green with dark blotches (young). Side of head and flank-silvery, often with golden sheen. And is smaller in size (common 5-30 cm, max. 40 cm) (Golani et al. 2002). The Smooth pufferfish *Sphoeroides pachygaster* is easily distinguished from the rest Tetraodontidae by its smooth skin. (figure 1). Juvenile individuals of *L. sceleratus* can be mistaken for *Lagocephalus suezensis* and/or *Torquigener flavimaculosus* (figure 1). However, *Lagocephalus suezensis* whose common size is 7-15 cm (max. 18 cm) bears 10 dorsal rays and irregularly shaped brown to grey dots of various sizes on its back, as opposed to the black distinct dots of *L. sceleratus*. The Yellowspotted puffer *Torquigener flavimaculosus* is even smaller in size [common 5-9 cm (max. 11 cm)] and is distinguished by its round

caudal fin.



Sphaeroides pachygaster

***Lagocephalus guentheri* (S. Kalogirou)**

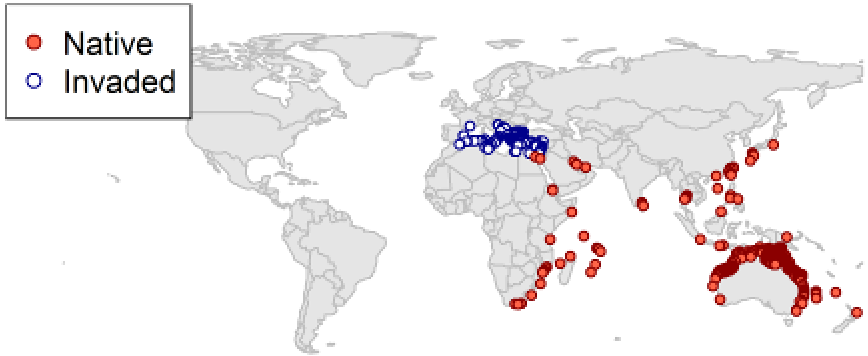
***Lagocephalus suezensis* (M Corsini-Foka)**

***Torquigener flavimaculosus* (M Corsini-Foka)**

Figure 1: representatives of the 4 alien species of Tetraodontoidea in the Mediterranean.

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

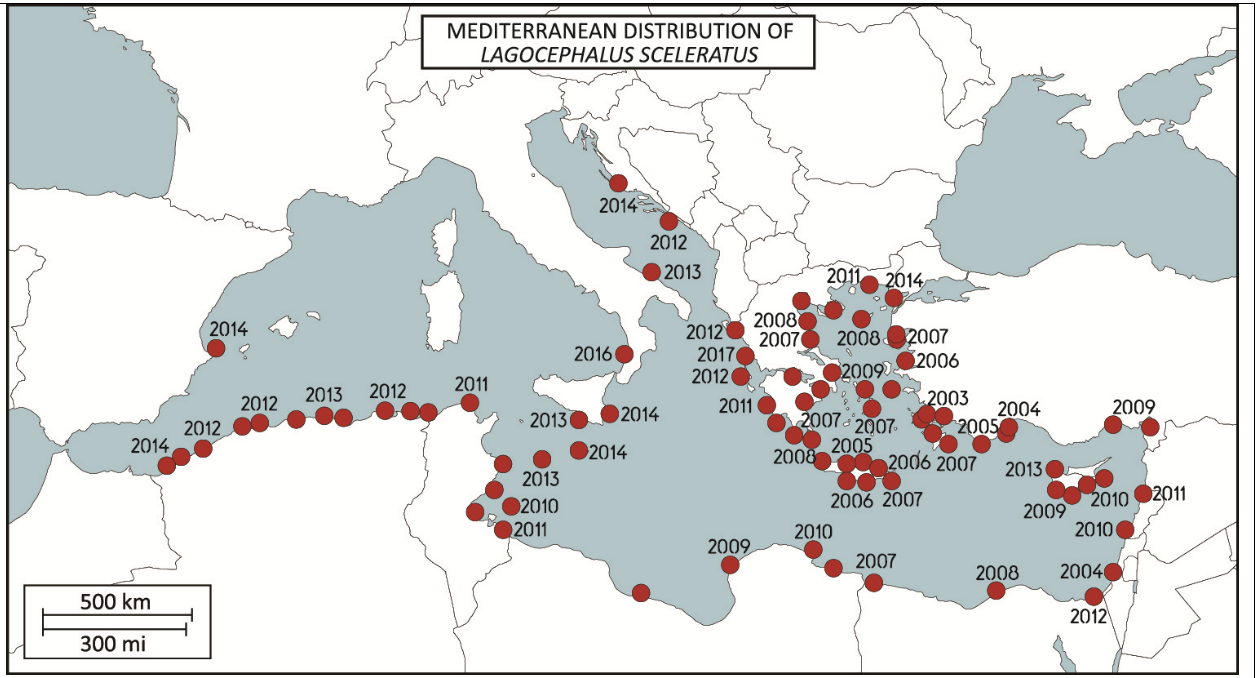
- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);

	• other alien species without similar invasive characteristics, potential substitute species; • native species, potential misidentification and mis-targeting
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	No
A4. Where is the organism native?	Tropical and sub-tropical waters of the Indo-West Pacific including the Red Sea (figure 2)  Figure 2: World distribution of *L. sceleratus* (data source: OBIS and HCMR database) *L. sceleratus* inhabits shallow coastal habitats, at depths generally between 0-180m. It spawns over shallow, vegetated habitats (e.g. seagrass beds) and recruits in sandy habitats but can also be found over muddy/mixed sediments, rocky bottoms and in the open sea. including the following elements:
A5. What is the global non-native distribution of the organism outside the risk assessment area?	Lessepsian immigrant to the Mediterranean Sea See invasion history in 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?	Recorded: Marine regions: • Mediterranean Sea • Black Sea (Sea of Marmara, outside the Risk Assessment area) Marine subregions:

	• Western Mediterranean Sea • Adriatic Sea: 2012 (Sulić-Šprem et al., 2014) • Ionian Sea-Central Mediterranean Seas: 2012 (Jribi & Bradai, 2012) • Aegean-Levantine Sea: 2003 (Turkey: Filiz & Er 2004/ Akyol et al, 2005); 2004 (Cyprus: DFRM, 2006) Established: Marine regions: • Mediterranean Sea Marine subregions: • Western Mediterranean Sea • Adriatic Sea • Ionian Sea, Central Mediterranean Sea • Aegean-Levantine Sea
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?	For details see ANNEX VIII – Results of the Habitat Suitability Model. Current climate: List regions • Western Mediterranean Sea, Adriatic Sea, Black Sea (Sea of Marmara), Iberian and Bay of Biscay Future climate: List regions • Western Mediterranean Sea, Adriatic Sea, Black Sea (Sea of Marmara), Iberian and Bay of Biscay For the assessment of future distribution both the RCP 4.5 and RCP 8.5 scenarios were considered in the species distribution model and the model was run with 2050 and 2100 forecasted data. Mean Sea

	Surface Temperature (SST) and maximum temperature at maximum depth were the two climate change related parameters most important for future establishment. In Europe, nearly all of the Mediterranean coastal region was predicted to be suitable for establishment with higher suitability in the eastern Mediterranean than the west because of cooler sea surface temperatures. Limited establishment at the Atlantic coast of southern Spain and Portugal is possible, but low sea surface temperature is predicted to prevent northwards spread into the Atlantic. Invasion of the Black Sea beyond the Sea of Marmara was predicted to be prevented by low temperature although the Black Sea also has very low salinity, which is expected to act as a limiting factor for establishment. By the 2050s, under both climate change scenarios, suitability in the Mediterranean increases, but the model predicts little potential expansion of the suitable region into the Atlantic or the Black Sea. For details on the assumptions made in relation to climate change see annex VI: projection of climatic suitability.
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.	Recorded in the following Member States: Cyprus: 2004 (DFMR, 2006) Greece: 2005 (Corsini et al., 2006) Malta: 2014 (Deidun et al., 2015) - casual Italy: 2013 (Azzurro et al. 2014) Croatia: 2012 (Sulić-Šprem et al., 2014) Spain: 2014 (Izguerdo-Munoz & Izguerdo-Gomez, 2014) – casual Established: List member states Cyprus: since 2007-Katsanevakis et al., 2009 Greece: Peristeraki et al., 2006 Italy: Azzurro et al., 2016a Croatia: Joksimović & Dulčić, 2016 in Karachle et al., 2016 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. Invasion History One of the 'worst'invaders of the Mediterranean Sea (Streftaris and Zenetos 2006), the silver-cheeked toadfish *Lagocephalus sceleratus* has exhibited a remarkable westward and northward expansion within the Mediterranean Sea since its first record from Gökova Bay, south Turkey, in 2003 (Filiz & Er 2004/ Akyol et al., 2005). Subsequently, it established large populations along the coasts of the Levantine Sea

	such as Israel (2004: Golani & Levy, 2005), Cyprus (2004: DFMR, 2005), Lebanon (2005: Carpentieri et al., 2009; Nader et al., 2012), Greece (2005: Corsini et al., 2006; Kasapidis et al., 2007), Egypt (2006: Halim & Rikzalla, 2011), Libya (2006: Kacem-Snoussi et al. 2009), and Syria (2012: Khalaf et al., 2014; Galiya et al., 2015). In the Aegean Sea the species was detected as early as 2003 along the Turkish costs (Bilecenoglu et al., 2006), and it was found widespread by 2005 (Kasapidis et al., 2007; Peristeraki et al., 2006). By 2009 *L. sceleratus* had established in the Ionian coasts of Greece but it took 7 years before a first observation was made in the Italian Ionian (Azzurro et al., 2016a). Within the central Mediterranean *L. sceleratus* had reached the Straits of Sicily by 2010 (Jribi and Bradai 2012) and spread rapidly along the Tunisian coast (Ben Souissi et al. 2014). According to Ounifi-Ben Amor et al. (2016) *L. sceleratus* is well established along the Tunisian coast. Azzurro et al. (2014) reported its finding off Lampedusa Island in 2013, while in January 2014 *L. sceleratus* was observed off the eastern coast of Sicily (Tiralongo and Tibullo in Kapiris et al., 2014). Šprem et al. (2014) document the northernmost record of the species from the Adriatic Sea (Croatia). In the southern Adriatic Sea there are a few records from Montenegro (Joksimović & Dulčić, 2016 in Karachle et al., 2016; Azzurro et al., 2018), and Italy (Azzurro et al., 2018). With regards to the western Mediterranean, there are sporadic records in Spain (Izguerdo-Munoz & Izguerdo-Gomez, 2014), and Italy (Tyrrhenian Sea: Azzurro et al., 2016a). In contrast *L. sceleratus* appears to be established and widespread in Algeria (Grimes et al., 2018), where it was detected in 2012 (Refes & Semahi, 2014; Kara et al., 2015) and Tunisia (Ben Souissi et al., 2014).

	 Figure 3. Mediterranean distribution of *L. sceleratus*
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: see Annex VIII. Current climate: Cyprus, Greece, Italy, Croatia, Malta, France, Slovenia, Spain, Portugal Future climate: List member states Cyprus, Greece, Italy, Croatia, Malta, France, Slovenia, Spain, Portugal For the assessment of future distribution both the RCP 4.5 and RCP 8.5 scenarios were considered in the species distribution model and the model was run with 2050 and 2100 forecasted data. Mean Sea Surface Temperature (SST) and maximum temperature at maximum depth were the two climate change related parameters most important for future establishment.

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?	*L. sceleratus* has become a major member of the ichthyofauna of the eastern Mediterranean wherever it has become established (Israel, Lebanon, Syria, Turkey) and in some regions it dominates the biomass of fish populations (especially in recent years, when older and larger individuals are increasing in abundance) – see Q2.13 and 2.14 in the Impacts section for details. By virtue of its powerful jaws and characteristic teeth structure of four solid incisor, it can easily crush and consume almost all species of benthos, which form part of its diet. In particular, it preys on native cephalopods and crustaceans, purportedly causing declines in the populations of these species (as inferred from fisheries catches). Apart from the effects of direct voracious predation on almost all benthic fauna, it also competes to a high degree with all other benthic-feeding species for food (Galanidi et al., 2018 and references therein).
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	Marine regions: • Mediterranean Sea Marine subregions: Aegean-Levantine Sea Fisheries data from Cyprus, including pre- and post-immigration data, indirectly show measurable declines in the abundances of native cephalopod species (Michailidis, 2010; CPUE data from Cyprus DFMR Annual Reports for the years 2001 to 2013 – see ANNEX XI). It is important to note however that causation for these declines has not been clearly identified. Its high abundance in coastal fish communities of the Dodecanese area (Greek Islands) combined with ecological and social impacts (see section 'Magnitude of Impact', p.42 for detailed accounts), clearly classify *L. sceleratus* as a pest for fisheries and a potential threat for biodiversity (Kalogirou, 2013). Its preference for *Posidonia oceanica* beds for spawning (Kalogirou et al., 2010) threatens the nursery value of these habitats, where large mature *L. sceleratus* can exert heavy predation pressure on juvenile fish and invertebrates. There is no evidence to suggest however that the structure of *Posidonia* meadows would be affected.
A12. In which EU member states has the species shown signs of invasiveness?	[delete as appropriate] **Cyprus**: "After its first recording in Cyprus in 2004 *Lagocephalus sceleratus* has become a dominant species in many waterbodies of the island, probably because it is a fast-growing species, it is capable of reproducing at only 2 years of age, it is not commercially harvested and has no known predators in the area. Today it is considered an invasive species in Cyprus, due to its suspected impacts on cephalopod populations (Q.A11), as well as its impacts on fisheries and human health (The species is known to damage both the fishing gear and the catch of the fishermen with its powerful jaws and it contains a powerful neurotoxin (TTX) which can cause serious poisoning, even death, if consumed).

	Greece: *L. sceleratus* persists in the Rhodes area, appearing abundant in certain cases and causing damage to fishing gears and commercial catches. Studies conducted in 2009 revealed that *L. sceleratus* was present in three of four sets of trammel nets, resulting in 26% of total biomass (Corsini-Foka & Pancucci-Papadopoulou, 2010). This ratio has climbed to 63% in two of three sets of trammel nets, studied in November 2015, (M.Corsini-Foka, unpublished data). High abundances, combined with dietary information and native species population declines (anecdotal reports from fishermen and unpublished data from the local Sea Fisheries Committee, Corsini-Foka, M and Perrakis, E., pers.comm, January 2018) indicate strong predation impacts. *L. sceleratus* is extremely abundant today around Crete, where the frequency of occurrence in fisheries catches in 2017 reached 57% (N. Peristeraki, HCMR unpublished data), and is dominated by ever increasing in age and size individuals. Declines of native cephalopod and fish populations are also suspected (e.g. Panagopoulou et al., 2017) but inference is not based on systematically collected data. In 2017, the fishing industry in Crete reported up to 800 kg of *L. sceleratus* per vessel per day of fishing. The study of the reproductive pattern in the Crete region shows that there are reproductive fields of the species in the waters around Crete, with more pronounced reproductive activity between June and July. In 2018, a large number of coastal fishermen in Crete are ready to withdraw their vessels and abandon commercial fishing because they cannot withstand the economic damage caused by the pufferfish (Nota Peristeraki, HCMR pers. communication).
A13. Describe any known socio-economic benefits of the organism.	*Lagocephalus sceleratus* is incidentally harvested for human consumption in parts of its native and invasive range (Shao et al., 2014). The species has received considerable attention from the public and the scientific community as it contains high concentrations of tetrodotoxin (TTX) in its tissues, which can be fatal when consumed (Kosker et al., 2016). The tetrodotoxin is a potent neurotoxin responsible for many human intoxications and fatalities each year. However, due to its paralysis effect, this neurotoxin could be used in the medical field as an analgesic to treat some cancer pains (Lago et al., 2015). New clinical studies suggest that low-dose TTX can safely relieve severe, treatment-resistant cancer pain. [USA: Joshi et al., 2006; Canada: Hagen et al., 2008]. The therapeutic potential of TTX in addiction is supported by studies in laboratory animals. Clinical studies suggest that low-dose TTX is acutely effective in reducing cue-induced increases in heroin craving and associated anxiety (Shi et al., 2009). Currently, the synthetic production of TTX is considered more cost-effective and reliable than production from harvested fish (Yu, 2007; Lago et al., 2015; see also Management Annex).

	In the Persian Gulf, *Lagocephalus sceleratus* is of no interest to the fisheries industry but is referred to as commercially collected and sold in the aquarium trade (Scott *et al*. 1974). Recent information indicates that the species in fact is not in the aquarium trade (Tracey King, OATA, pers.comm – see also Q1.1).

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.”
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[8] and the provided key to pathways[9].
- 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.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- 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 introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment	3	high	CORRIDOR -introduction via the Suez Canal is the primary pathway of introduction into the Mediterranean Sea - recipient countries are located in the Levantine basin (Turkey, first record in 2003 / Egypt, first record in 2005, Israel, first record in 2004 – see QA.8 for

[8] https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf
[9] https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf

section)			invasion history) UNAIDED Natural dispersal across borders from neighbouring countries, where the species has been introduced (See QA.xx for a full list). This is the main introduction pathway into the RAA and for further introductions into currently uninvaded regions of the RA area. Ballast water transport has not been considered as a pathway of introduction (or spread) because the pattern of spread of *L. sceleratus* in a gradual progression from the Suez Canal towards the north-east Mediterranean and along the north coast of Africa simultaneously (see Invasion history and map in QA.8) strongly indicate unaided dispersal. The lack of records of Tetraodontidae species from ballast water (Wonham et al., 2000) further supports this hypothesis (Bañón & Santás, 2011). Ballast transport may be happening to some degree but, compared to the propagule pressure exerted by already established neighbouring populations it is expected to be negligible. ESCAPE FROM CONFINEMENT (Botanic gardens / zoo / aquaria) *L. sceleratus* is referred to as an aquarium species in a number of databases (FishBase, IUCN), citing as a primary reference Scott et al., (1974). However, a search through online pet and aquarium supply stores returned no results for *L. sceleratus* as an aquarium trade species. Additionally, a request for aquarium trade sales data from the Ornamental Aquarium Trade Association (OATA) revealed that the species is not in trade by the ornamental aquatic sector in the UK and most likely not in the rest of the EU either (Tracey King, pers.comm.). On the other hand, *L. sceleratus* is currently displayed in public aquaria in Greece, Cyprus and Egypt (Alexandria), while in the past it has been on

			display in one more public aquarium in France (Corsini-Foka et al., 2014; European Union Aquarium Curators (EUAC), pers.comm June 2018 – see Annex IX for full list of EUAC respondents). ESCAPE FROM CONFINEMENT (Research and ex-situ breeding) *L. sceleratus* is used for bioprospecting studies of its venom (see A.13) in various laboratories around the world and such studies/applications have been proposed for the Mediterranean invasive populations as well as a potential management measure to reduce populations of the species (e.g. Kosker et al., 2016; Nader et al., 2012). Nevertheless, to our knowledge, no such efforts have been initiated in the invaded range to date (Turan et al., 2017) and the synthetic production of TTX, either chemosynthetically or through microbial synthesis, is considered to provide a more stable, reliable and cost-effective method than the extraction from harvested pufferfish (Yu, 2007; Lago et al., 2015). As such, this pathway is not considered currently active and was not fully assessed.
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (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. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	CORRIDOR (Suez Canal) UNAIDED (natural dispersal from neighbouring countries) ESCAPE FROM CONFINEMENT: Botanic gardens /	very likely very likely unlikely	primary pathway secondary pathway primary pathway- public aquaria/Unintentional

	zoo / aquaria		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).
Pathway name:	CORRIDOR (Interconnected waterways, basins & seas – Suez Canal)		
1.3a. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	unintentional	high	
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	likely	medium	*Lagocephalus sceleratus* is native and present in the Suez Gulf and all along the Red Sea, where it sustains a commercial (albeit illegal) fishery (Sabrah et al., 2006). The adults are highly mobile (Kalogirou, 2013; Coro et al., 2018) and swim in schools and, given the established populations at both ends of the Suez Canal, may even be part of the resident canal fauna (authors' judgement). The fecundity of the species is high (at least 625000–800000 eggs per year – see Q1.22 for estimates) and larvae & juveniles can stay in the water column for up to 2-3 months (Leis, 1991). However, because spawning of *L. sceleratus* occurs in the summer, when the, generally northward, net water transport in the canal changes to a net transport from the Mediterranean to the Red Sea (particularly in the late summer/early autumn months – Galil, 2006; Zakaria,

			2015 and references therein), it is considered less likely that large numbers of larvae will travel along this pathway over the course of the year. Even if eradicated in the Mediterranean Sea (both inside and outside the EU) the likelihood of reinvasion in the first recipient countries (Egypt, Israel) and hence its spread in the rest EU countries is very high.
1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	high	The organism has already successfully crossed the Suez Canal in numbers sufficient for successful establishment in the Mediterranean Sea. The current environmental conditions of the canal (after numerous expansions) offer suitable conditions for survival both of the adults (e.g. soft sediments, temperature and salinity ranges within the tolerance limits of *L. sceleratus*) and for the passage of larvae (e.g. current speeds that allow their passive passage through the Canal, given their extended pelagic phase – see Q1.22) (Galil, 2006; Katsanevakis et al., 2013).
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	high	There are currently no management practices that can prevent the survival of marine invasive species during their passage along the Suez Canal (Galil et al., 2017).
1.7a. How likely is the organism to enter the risk assessment area undetected?	NA		The organism will not enter the risk assessment area directly through this pathway but through natural dispersal from neighbouring countries. Given that the first record in the Mediterranean was from Turkey in 2003, the organism did remain undetected for a considerable time after its passage through the Suez Canal. New introductions through this pathway will likely be indistinguishable from already established populations, unless genetic studies are conducted.
1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?	likely	high	Adults can cross the Suez Canal at any time of the year. Spawning in the Gulf of Suez takes place in late spring and summer (Sabrah et al., 2006), thus larvae and

			juveniles may cross until June, when the direction of the current turns southerly at the north end of the canal and are then trapped in the canal or are moved southward in the late summer/autumn (Galil, 2006).
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very likely	high	During natural dispersal, organisms usually arrive and settle in suitable habitats or move on. *L. sceleratus* recruits on soft-sediment habitats – particularly sandy habitats - (Kalogirou, 2013), which are ubiquitous in the Mediterranean Sea (inside and outside the RA area) and is found in various different habitats (sandy, muddy, rocky, seagrass beds) at different life stages – see Q1.15 for details).
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	NA (RA area) very likely (neighbouring countries)	high	The organism will not enter the risk assessment area directly through this pathway but through natural dispersal from neighbouring countries. New introductions via this pathway are very likely and may increase the genetic diversity of the invasive populations with implications for further adaptability and spread. It has already entered the Mediterranean through the Suez Canal and has established successful populations in the East and Central Mediterranean.
End of pathway assessment, repeat as necessary.			
Pathway name:	UNAIDED (natural dispersal)		
1.3b. 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)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	unintentional	high	Since it was first reported in the Mediterranean (Turkey), the species has been recorded in neighbouring countries (Cyprus, Greece, Lebanon, Syria, Egypt, Tunisia, Italy, Malta, Croatia), hence its natural dispersal in marine (sub)regions of the risk assessment area is confirmed. (see also Invasion history – Q A.8)
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year?	very likely	high	The organism has already entered the RA area unaided and has established populations, reaching high densities, especially in the East Mediterranean (Q A10-12).

Subnote: In your comment 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.			Both adults (highly mobile, demersal predators) and larvae, with an extended pelagic phase (Q1.4a, Q1.22) can travel along this pathway. Extensive current establishment, high fecundity and an extended pelagic phase (see Q1.22) create high propagule pressure with a high potential for natural dispersal.
1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	high	The current natural dispersal of *L. sceleratus* from the Suez Canal to the Levantine coast and into the Central and West Mediterranean is unequivocal evidence that the species is able to survive and reproduce along this pathway, establishing populations along the way at suitable habitats and depths. (see Invasion history Q A.8)
1.6b. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	high	No management practices are in place concerning natural dispersal that can affect the organism's ability to survive in the RA area. Early detection systems could and do operate through official and unofficial networks of national experts with local stakeholders (e.g. Azzurro et al., 2016a), but would not be of use to prevent survival.
1.7b. How likely is the organism to enter the risk assessment area undetected?	likely (early life stages)	high	See Q1.7a for natural dispersal between the Suez Canal and first record in Turkey The current level of awareness if this toxic invader has substantially decreased its likelihood of remaining undetected in the case of new introductions (e.g. see Invasion history for its rapid detection in Levantine countries following the first record, reported in 2004 by Filiz & Er 2004/ Akyol et al., 2005). At the moment early detection systems operate through official and unofficial networks of national experts with local stakeholders and through official authorities in

			many Mediterranean countries (SeaWatchers, ELNAIS, iSea, relevant competent Authorities). This has contributed to the early detection of the species as it expands its range, as attested by the continuous reporting of casual records in the Adriatic (e.g. Azurro et al., 2016a), Malta (Deidun et al, 2015; Andaloro et al., 2016), the Sea of Marmara and the western Mediterranean. However, the probability of observing an introduction event at the larval or early life stages is rather low and such introductions would most likely remain undetected, especially since the juveniles of *L. sceleratus* can be misidentified for *Spicara smaris, Boops boops* and *Atherina hepsetus* (Katikou et al., 2009; Kalogirou, 2013) if fishermen are unfamiliar with the species.
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	high	Adults can travel from neighbouring populations at any time of the year but reproductive cohorts are more likely to migrate to shallow areas to spawn in the early summer (see Q1.15 for habitat use). Larvae and juveniles settle to soft-sediment habitats 1-3 months later (Q1.22).
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very likely	high	See Q1.9a, Q1.15
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very likely	high	The organism has already entered the RA area, is continuously expanding its range throughout the Mediterranean through natural dispersal. Further introductions through this pathway are expected (see Risk of Establishment section and Annex XX for Habitat Suitability Model).
End of pathway assessment, repeat as necessary.			
Pathway name:	ESCAPE FROM CONFINEMENT (Botanic gardens / zoo / aquaria)		
1.3c. Is introduction along this pathway intentional (e.g.	unintentional	high	There is a low likelihood that the species can escape

the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)			from public aquaria based near the sea.
1.4c. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment 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.	unlikely	high	A query was addressed to the European Union Aquarium Curators (EUAC) members and other Aquarium curators, about the display of *L. sceleratus* (see Annex IX). Out of 114 recipients, 34 replied. Of those, 2 mentioned that *L. sceleratus* is currently on display. These are the Createaquarium and the Rhodes Aquarium, both in Greece. In one more case, the species was displayed in the Marineland Parcs-Antibes Cedex Aquarium in France in 2014. Additionally, *L. sceleratus* is displayed in the Alexandria Aquarium (Egypt) and the Ocean Aquarium in Cyprus (Corsini-Foka et al., 2014). For the Mediterranean aquaria, specimens displayed were collected from invasive populations in the wild, where *L. sceleratus* is already established (Corsini-Foka et al., 2014). In any case if an accidental escape occurs, it will be only small numbers of the organism that travel along this pathway, compared with the naturally dispersing populations.
1.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	medium	Favourable conditions inside the tanks and in the surrounding environment can facilitate the organisms' survival.
1.6c. How likely is the organism to survive existing management practices during passage along the pathway?	unlikely	high	Article 3 of the EU Zoos Directive recognizes that for aquatic species, it is paramount to prevent incidental

			escapes from the water. A first line of actions is to secure enclosures against animal escape. In large public aquaria, circulation systems are closed. The recirculated water in the tanks is continuously filtered and disinfected (UV, ozonation, skimmers, etc.). At regular intervals, a part of the seawater is renewed. The water changed and discharged outside, is always subjected by law to strictly disinfection and filtration before outlet (both for coastal and inland aquaria). Consequently, assuming compliance with regulations, the probability that eggs or larvae survive is zero. Also, all equipment should be disinfected, mainly for parasites and diseases. However, in public small open or semi-open circulation system aquaria displaying tropical organisms, if the outlet is in the sea or near the sea and is not subjected to control, or cleaning equipment is not appropriate, there is some probability to discharge eggs or larvae. *L. sceleratus* in the Rhodes Aquarium has been on display in both open and closed circulation systems (Corsini-Foka et al., 2014). In small aquaria displaying to the public a series of tropical small fish they use LSS, Life Support System (Biological and mechanical filter, UV lamp and skimmer). Accidental escape or cleaning operation without appropriate disinfection may be a risk.
1.7c. How likely is the organism to enter the risk assessment area undetected?	very likely	high	If an escape from aquaria occurs, it can be expected that specimens show up in marine habitats. This could be expected for all the aquaria currently displaying the species, as they are located in the vicinity of coastal cities in the Aegean-Levantine subregion. Considering that *L. sceleratus* is widely established in this area, new introductions will be indistinguishable from existing populations and of minor importance.
1.8c. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	low	It depends on the frequency of cleaning operations. Considering the large size of the species and the high

			numbers of eggs and juveniles produced, it would be the early life stages that are most likely to enter the RAA through this pathway.
1.9c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	low	Such an event is possible if the organism escapes from a facility that operates and has an outlet in or near the sea without appropriate management practices. No information was found on incidents of escape of *L. sceleratus* different life stages from aquaria.
1.10c. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	unlikely	medium	*L. sceleratus* is or has been displayed in a small number of large public aquaria in Mediterranean countries, while information on small public and private aquaria indicates that the species is not in trade by the ornamental aquatic sector in the UK and most likely not in the rest of the EU either (Tracey King, OATA, pers.comm.). The probability of escape of propagules is considered low, as in only one occasion the species is documented to be kept in open circulation systems. Considering that *L. sceleratus* is widely established in the vicinity of these aquaria, new introductions will be indistinguishable from existing populations and minor in importance.
End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	very likely	high	*L. sceleratus* entered the Mediterranean Sea through the Suez Canal, a pathway that is still active and will likely provide opportunities for additional introductions. The species is already established in the RA area through natural dispersal from the recipient neighbouring countries and is continuously expanding its range throughout the Mediterranean. Further introductions through this pathway are expected in currently uninvaded regions. The likelihood of introduction through escape from public aquaria is considered to be low.

1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	very likely	high	For future climate change predictions, RCP 4.5 and 8.5 scenarios were taken into account (see Annex VIII for habitat suitability model). The main pathway of introduction that will be affected by foreseeable climate change conditions, particularly sea surface and bottom temperature, is natural dispersal. Future increases in temperature are expected to increase the likelihood of introduction (and spread) in areas of the Mediterranean Sea which currently offer less favourable climatic conditions for winter survival and summer spawning, such as the North Adriatic, the Gulf of Lyon and the Sea of Marmara.

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. If the species is established in all Member States, continue with Question 1.16.

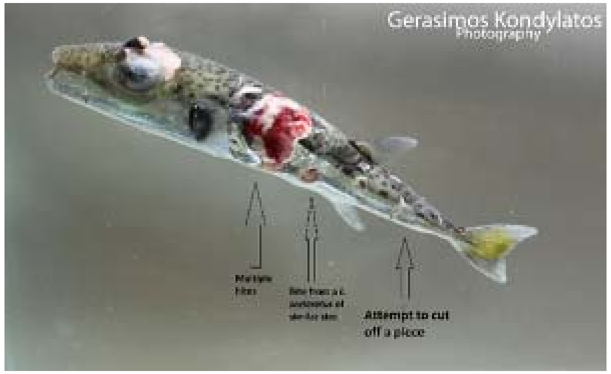
QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very likely	high	The species is already established in the RA area, with abundant populations in the Eastern Mediterranean and an expanding distribution to the northern and western basins of the Mediterranean. For the purpose of this Risk Assessment, a study was conducted to project the climatic suitability for potential establishment of *Lagocephalus sceleratus* in Europe, under current and predicted future climatic conditions. Temperature variables chosen for the model were 1) Maximum temperature at maximum depth (°C) since

			spawning may be limited by low temperature (see Q1.22) and 2) Mean sea surface temperature (°C) which may represent a constraint on adult and juvenile survival. Juveniles in particular persist in coastal areas throughout the year. The model results showed that both parameters are important predictors of habitat suitability (especially maximum temperature at maximum depth) and that *L. sceleratus* finds suitable conditions for establishment throughout most of the Mediterranean Sea coasts and into Atlantic waters as far as southern Spain and southern Portugal along the Iberian Coast (SEE APPENDIX). Additionally, literature on the con-generic *Lagocephalus lunaris spadiceus* (Fujita, 1966) and the distribution of spawning aggregations of *L. sceleratus* in southern Cyprus (Michailidis, 2010; Rousou et al., 2014) indicate that there might a thermal limit for spawning at around 21-22 °C sea surface temperature (SST) in June (peak spawning month). Short-lived upwellings which reduce the SST by 4-5 °C during the summer months were suggested to be responsible for the absence of spawning aggregations from the southwestern coast of Cyprus, where immature fish were consistently present (Rousou et al., 2014). A threshold of 21.7 °C SST for the month of June was tentatively applied as a limiting factor for spawning (value taken from Fujita, 1966) and the resulting map indicated that certain areas in the western Mediterranean may not favour the reproduction of the species. Since this value may not accurately represent the physiological requirements of *L. sceleratus* in the RA area, it

			could be rather perceived as a relative measure of recruitment strength, as it is considered unlikely that a species will thrive in large numbers at the boundaries of areas of habitat suitability even though it may be present (Townhill et al., 2017).
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	very likely	high	The species is already established in the RA area. Salinity requirements, according to native presence records (OBIS) range between 29-40 psu (MARSPEC dataset, Sbrocco & Barber, 2013). The different modelling algorithms employed resulted in variable response plots to salinity and a rather low predictive power for this parameter. However, salinity is expected to act as a barrier for the two enclosed low-salinity basins of the RA area (i.e. Black Sea with salinity values 14-18 psu, and the Baltic Sea, excluding the Kattegat, where SSS<20psu), but not be as important in fine-tuning potential establishment projections for the rest of the RA area.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	widespread	very high	*L. sceleratus* occupies shallow coastal habitats generally between depths of 0-180m, even though it has been recorded from 250m in the Red Sea (Baranes & Golani, 1993) and from 350-400m off Spain (Izguerdo-Munoz & Izguerdo-Gomez, 2014). The species recruits to sandy habitats in the summer, while an ontogenetic shift to *Posidonia oceanica* beds occurs close to maturity (>30cm) (Kalogirou, 2013). *P. oceanica* beds are preferably used as spawning habitats in the Mediterranean in the summer but spawning grounds are abandoned in autumn by the reproductive cohorts (Kalogirou, 2013; Rousou et al., 2014), which are hypothesized to move to deeper waters (Michailidis, 2010, 2011; MRS, 1997) or rocky bottoms. *P. oceanica* meadows are endemic and

			widespread in the Mediterranean, while shallow, sandy habitats are ubiquitous in the RA area. Specific information about the spawning habitat of *L. sceleratus* in its native range is not available, however members of the Tetraodontidae family are demersal spawners (Leis, 1991) which cement their eggs to stones and rocks, bury them in the sand or lay them attached to algal fonds (Stroud, 1989 and references therein). The species *Fugu pardalis* is known to spawn over seagrass beds in Japan (Fujita, 1962). Shallow, vegetated habitats (e.g. seagrass beds other than *Posidonia*) are also widespread in parts of the RA area not already invaded by *L. sceleratus*.
1.16. 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?	NA		*L. sceleratus* does not require another species for completion of its life cycle, except for prey, which is abundantly available in the preferred habitats.
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very likely	medium	Studies documenting competitive interactions between *L. sceleratus* and native species or presenting evidence for competitive displacement of native species in the RA area were not found. Nevertheless, based on its successful establishment and population increase in the Mediterranean Sea, it is commonly assumed to lack competitors (EastMed, 2010). The analysis of the ecological niche space (as inferred from morphological space) of the receiving indigenous fish communities demonstrated a small resident assemblage of a similar guild, indicating high niche opportunities (Azzurro et al., 2014).
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the	likely	medium	*L. sceleratus* is generally considered to have few natural enemies, both in the native and invaded

risk assessment area?			range (East Med, 2010). One reason might be the unpalatability of the eggs and larvae of the Tetraodontidae family (Gladstone, 1987) due to their TTX content. Gladstone (1987) found that potential egg predators (reef fishes in laboratory experiments) first mouthed and then immediately egested both eggs and larvae of the con-familiar *Canthigaster valentini*. Another predator avoidance mechanism is the ability to inflate its body by swallowing water (Nader et al., 2012). Nevertheless, pufferfish species in their native range are reported to be consumed by large predators such as *Rachycentron canadum, Scomberomorus commerson* and skipjack tuna *Katsuwonus pelamis* (Mohamed et al., 2013 – Arabian Sea). The first two are present in the Mediterranean Sea as alien species and the third is native and widespread in the RA area. Predation of these species on *L. sceleratus* specifically is not known such that they are assumed to not represent any significant potential threat to the establishment of the species in the RA area. In the Mediterranean, Kleitou et al. (2018) recently reported an incidence of *L. sceleratus* juvenile predation by the common dolphinfish *Coryphaena hippurus* over shallow coastal waters of Crete (Greece). While *C. hippurus* is known to consume a variety of Tetraodontidae species in the Pacific and Atlantic Oceans and the Arabian Sea (e.g. Varela et al., 2016 and references in Kleitou), this was the first report of a Tetraodontidae in the species' diet for the Mediterranean Sea (Kleitou et al., 2018), thus the scope for natural control of *L. sceleratus* due to *C. hippurus* predation is still unclear.

			Cannibalism has been observed at the Rhodes Aquarium (photo from tank) and when feeding in fishers' nets (DFMR Cyprus, 2012, information brochure).  Natural control may also be exerted by the ectoparasitic isopod *Gnathia* spp., whose larvae was identified from one specimen of *L. sceleratus* from the south-eastern Aegean, causing severe damage to the gills (Bakopoulos et al., 2017). This assumption however is based on the results of one study only (n=41), where prevalence of *Gnathia* spp. infection was low (2.4%); more studies on the effects of parasitism on *L. sceleratus* are needed to make stronger inference.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very likely	very high	Early detection systems operate through official and unofficial networks of national experts with local stakeholders (e.g. Azzurro et al., 2016a), but would not be of use to prevent establishment. Intensive targeted fishery in combination with a bounty system has been in place in Cyprus since 2012, years after the species had already attained high populations in the region and had spread and

			established in neighbouring areas. The measure does not seem to effectively control populations in Cyprus (N. Michailidis, pers. comm.); its efficacy if implemented at an earlier time in the establishment of the species is uncertain. Despite the ban on fishing, selling and marketing of the species in many Mediterranean countries, there are no guidelines for the disposal of the fish after capture (e.g. ban on release) that could affect further establishment and spread (V. Karachle, pers. comm.).
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	likely	medium	The EU Regulation 1967/2006 bans trawling in the Mediterranean Sea at depths shallower than 50 m throughout the year and will afford protection to *L. sceleratus* populations which are particularly abundant in shallow water assemblages (Kalogirou 2013, Corsini-Foka et al., 2010). Additional fisheries restrictions implemented nationally in EU countries, mostly in the spring and summer months (for a comprehensive review and data collation see the MEDISEH project – http://imbriw.hcmr.gr/en/mediseh/), will protect the spawning aggregations of the species and may facilitate establishment.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	very likely	high	Spawning in shallow, vegetated areas renders possible eradication measures (e.g. by trawling) destructive for native species and habitats and thus highly impractical, especially in the case of *P. oceanica* meadows. High fecundity and an extended pelagic phase (see Q1.22) can create high propagule pressure with a high potential for spread.
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	very likely	medium	Information on the larval development of *L. sceleratus* is inferred from studies on con-familiars /congeners. *L. sceleratus* has demersal eggs and

			pelagic larvae (Leis, 1991). Fujita (1966) described the larval development of artificially hatched and reared larvae of *Lagocephalus lunaris*. Hatching occurred after approximately 72 hours at 21.7-24.5 °C. Larvae under laboratory conditions reached the early juvenile stage (6.6mm length) after 26-31 days. The Tetraodontidae family also have pelagic juvenile stages (Leis, 1991) that can prolong their presence in the water column for up to 2-3 months, e.g. *Canthigaster valentini* has a minimum settlement age of 64 days and can extend it up to 113 days (Stroud, 1989), while Fujita (1966) reported the collection of 12mm long *L. lunaris* juveniles from surface waters in Japan and even the capture of one individual at the early fish stage (28mm length). Conversely, there have been a number of studies on Mediterranean populations, documenting spawning and fecundity characteristics of the species. In the Mediterranean *L. sceleratus* achieves maturity at around 2 years of age (Farrag et al., 2015; Michailidis 2010) and sizes ranging from 36cm in Rhodes (Kalogirou, 2013) to 48.8cm in Cyprus (Rousou et al., 2014). It has an early summer spawning period, peaking in June throughout the Eastern Mediterranean (Aydin, 2011; Rousou, 2014, Farrag et al., 2015, Michailidis, 2010) and the Gulf of Suez (Sabrah et al., 2006). The relative fecundity was estimated as 776±231 eggs/g total body weight in Egypt (Farrag et al., 2015) and 780.8±171.8 eggs/g total body weight in Turkey (Aydin, 2011). Considering that a mature female can weigh upwards of 800-1000g (e.g. see Length-Weight curves in Aydin, 2011), it can produce at least 625000–800000 eggs per year.

1.23. How likely is the adaptability of the organism to facilitate its establishment?	likely	high	*L. sceleratus* is generally believed to be adaptable to colder temperatures in the invaded range compared with areas of its native distribution (Manal et al., 2012). However, there are already some isolated records from south Africa and southern Australia from waters with rather cold temperatures (below the 16 °C threshold we established as unsuitable for establishment in the model – in the modelling annex it is stated that 1% of the occurrence records fall into this unsuitable area.). Thus, it is possible that some populations are already tolerant. Genetic evidence from Turkey (Vella et al., 2017) and Greece (Giusti et al., 2018) indicates that the eastern Mediterranean populations are more closely related to East Africa individuals which have a higher likelihood of being tolerant to low temperatures. The establishment of the species in the Northern Aegean and a number of casual records in locations of the Mediterranean where mean annual surface SST is below 18 °C (BIO-ORACLE data set 2000-2014 - Assis et al., 2017) (i.e.two records in the Sea of Marmara (Artuz & Kubanc, 2015; Irmak & Altinagac, 2015 and one record from deep waters (350-400m) off the Spanish coast (Izguerdo-Munoz & Izguerdo-Gomez, 2014), where the temperature at the capture location and depth ranged between 12-13°C), corroborate the tolerance of *L. sceleratus* to lower temperatures. Further adaptation to cooler temperatures is likely to expand the potential invasive distribution, maybe allowing stronger colonisation of the western Mediterranean and along the southern Atlantic coast.
1.24. How likely is it that the organism could establish	likely	high	Based on the cytochrome c oxidase subunit 1 gene

despite low genetic diversity in the founder population?			sequence (COI), no haplotype diversity was found for *L. sceleratus* from Lebanon (Bariche et al., 2015, n=5) and Turkey (Yokes & Bilecenoglu, 2017, n=14; Vella et al., 2017, n=12), indicating a strong founder effect. Vella et al. (2017) note that "Nearly all the publicly available *L. sceleratus* GenBank COI records of Mediterranean origin share the same COI haplotype", in their samples however, they did find 6 haplotypes based on the mtDNA control region (CR) sequence, a molecular marker that evolves faster than COI and could prove useful for following up genetic population structure and connectivity of expanding species.
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	very likely	high	Already established in marine region/subregions of the RA area both within and outside RAA waters (see QA.8 for a detailed description of the invasion history from the Suez Canal to RAA waters and Q1.27 for the likelihood of further establishment).
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	very likely	high	Given the continuous propagule pressure from established populations in neighbouring areas, casual populations are very likely to continue to occur in habitats unsuitable for reproduction/establishment through natural dispersal.
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	very likely *Mediterranean Sea* moderately likely *Bay of Biscay and the Iberian Coast, Black Sea (Sea of Marmara)* unlikely *Baltic Sea, Greater*	 high low high	Based on the invasion history of the species in the Mediterranean, its abiotic requirements, the results of the habitat suitability model and the tentative thermal limit for spawning in the summer, *L. sceleratus* is considered very likely to establish further populations in the Western Mediterranean (currently only established in Algeria and Tunisia, which are outside the RA area), with higher probabilities of establishment along the western Italian coast, Sardinia, Corsica and the eastern part

	North Sea, Celtic Seas		of Mediterranean France, and a lower probability of establishment in the Gulf of Lyon and the Spanish Mediterranean coast, where temperatures close to the thermal limits of the species offer less favourable conditions both for the survival of the young fish and for prolific spawning. The North Adriatic is another area of the Mediterranean where the model predicts low habitat suitability, primarily due to low minimum SST, but considering the adaptability of *L. sceleratus* to cooler temperatures compared with its native range, it is possible that the model underpredicts this area. Along the Atlantic coast of Europe, the habitat suitability model predicts moderate likelihood of establishment only as far north as southern Spain and southern Portugal. Low temperatures will make establishment unlikely in the Greater North Sea and the Celtic Seas, while low salinities and temperatures will most likely prevent establishment in the Baltic Sea and the Black Sea. In terms of climatic conditions, the Sea of Marmara (which is outside of the risk assessment area) is a transition area between the Aegean and the Black Sea. In this region, 2 casual records have been observed since 2008, both close to the Dardanelle Straits and the prevailing conditions of the North Aegean, where the species is established. Thus, establishment in the Sea of Marmara is considered moderately likely, despite the low predicted suitability according to the model. As a general comment about the Species Distribution Model, a strong caveat on the predicted potential range is that spread towards the Atlantic, Adriatic and Black Sea is facilitating the

			movement of the species into novel environmental conditions (lower SST especially, to which *L. sceleratus* seems to be adaptable). Because these conditions are not experienced in other parts of the range, the model can't predict beyond the occurrence data and predict further niche expansion and may thus make an underprediction.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	very likely *Mediterranean Sea* moderately likely *Black Sea Bay of Biscay and the Iberian Coast* unlikely *Baltic Sea, Greater North Sea, Celtic Seas*	 high low high	For future climate change predictions, RCP 4.5 and 8.5 scenarios were considered (see Annex VIII). By the 2050s, under the moderate RCP4.5 and the extreme RCP8.5 climate change scenarios, suitability in the Mediterranean increases, especially in areas that were previously of low suitability (like the North Adriatic and the Gulf of Lyon), but the model predicts little potential expansion of the suitable region into the Atlantic. (See Annex VIII for habitat suitability maps and more details on model outputs). This may be attributed to some extent to underprediction by the model, but the local hydrographic conditions will likely also play a role. More specifically, frequent summer upwellings along the Portuguese coast that can reduce the sea surface temperature by 3-4 °C in the summer months for periods of 10-20 days (Vidal et al., 2017), may create a barrier for spawning (similar to what was observed in Cyprus by Rousou et al., 2014), even under increased SST conditions in a future climate scenario.

PROBABILITY OF SPREAD

Important notes:

- 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 spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	major	high	Following its first introduction in the Mediterranean via CORRIDOR, the species has spread to Cyprus and Greece unaided (Michailidis, 2010, Peristeraki, 2007). *L. sceleratus* is a large fish that can reach up to 110cm length and 7kg, has high fecundity, extended pelagic duration (larvae and juveniles), few predators and competitors and is showing signs of tolerance and possibly further adaptability to temperature conditions cooler than those in the bulk of its native range (see Q1.23). All these characteristics, along with favourable environmental conditions have contributed to the rapid spread of the species throughout most of the Mediterranean Sea.
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	minimal	high	The only human-assisted pathway relevant for *L. sceleratus* is ESCAPE from confinement from public aquaria and research facilities (see Q1.3c-1.10c). However, even if repeated escapes at separate locations do occur (which is unlikely), such events do not represent spread. Regarding the possibility of ballast water transport of larvae or juveniles, please see relevant comment in

			Q1.1
2.2a. 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 2.3 to 2.9 (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. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	UNAIDED (natural dispersal)		*L. sceleratus* has demersal eggs and pelagic larvae (Leis, 1991). Its rapid expansion to the Levantine only one year after its first detection (2003: Turkey) to Cyprus (2004) and Greece (2005) attests its invasion potential. For details see QA8: invasion history, Q1.3b-1.10b: Risk of Introduction-Unaided pathway Note: Most of the relevant information for this section has already been presented in the Introduction and Establishment sections, thus answers to the following questions will be brief, with reference to the appropriate questions and answers.
Pathway name:	UNAIDED: Natural spread		
2.3. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	unintentional	high	Since it was first reported in the Mediterranean (Turkey), the species has been recorded in neighbouring countries (Cyprus, Greece, Lebanon, Syria, Egypt, Tunisia), hence its natural dispersal in marine (sub)regions of the risk assessment area is confirmed.
2.4. 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?	very likely	high	Both adults and larvae can spread along this pathway. Extensive current establishment, high fecundity and an extended pelagic phase (see Q1.22, Q1.4a, Q1.4b) create high propagule pressure with a high potential for spread. The longevity and migration potential of the adults also contribute to the high potential for spread.
2.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)?	very likely	high	The current natural dispersal of *L. sceleratus* from the Suez Canal to the Levantine coast and into the Central and West Mediterranean is unequivocal evidence that

Subnote: In your comment consider whether the organism could multiply along the pathway.			the species is able to survive and reproduce along this pathway, establishing populations along the way at suitable habitats and depths. Moreover, it is showing signs of adaptability to temperature conditions cooler than those in its native range and is able to exploit favourable temperature conditions for rapid population increase and range expansion. Characteristically, the population explosion of the species during the summer of 2007 in the Aegean and its spread beyond the 14 oC isotherm was largely attributed to the anomalous high temperature observed in that period throughout Greece and the consequent production of unusual deeper warm water conditions (Pancucci-Papadopoulou et al., 2012), similarly to those observed for Rhodes Island (Corsini-Foka, 2010).
2.6. How likely is the organism to survive existing management practices during spread?	very likely	high	No management practices are in place concerning natural dispersal that can affect the organism's ability to establish in the RA area. Early detection systems could and do operate through official and unofficial networks of national experts with local stakeholders (e.g. Azzurro et al., 2016a), but would not be of use to prevent establishment. If a management practice such as intensive targeted fishery is to be applied, especially during the reproduction period, a reduction of probability to survive could be achieved, but this reduction is difficult to quantify and highly uncertain.
2.7. How likely is the organism to spread in the risk assessment area undetected?	likely (early life stages)	high	The expansion of *L. sceleratus* in the Mediterranean has generated an increasing concern about biodiversity protection and human health, and European countries were solicited to apply early warning measures. In Greece, fishermen have been warned by the Greek ministries of Health and Agriculture about the presence and associated risks of the toxic pufferfish *L. sceleratus*, soon after its arrival. Aiming to

			disseminate information, a fact sheet on *L. sceleratus*, containing a photo of the species, distinctive characters, and contact data was distributed to fishermen and citizen scientists (divers, anglers, port authorities) while this information has often been a front page on national and local newspapers. Similar awareness campaigns have been carried out in Cyprus. Awareness campaigns to manage the risk associated with the occurrence of *L. sceleratus* have been conducted by the competent governmental Institutions —ISPRA in Italy and the Department of Fisheries in Malta (Andaloro et al., 2016). Informative campaigns were launched in Italy and Spain soon after the first occurrences of *L. sceleratus* in these countries. Both the Spanish and Italian campaigns were promoted by the interactive web platform SEAWATCHERS www.seawatchers.org under the action 'invasive fishes' (Azzurro et al., 2016b). That being said, the probability of observing an introduction event at the larval or early life stages is rather low and such introductions would most likely remain undetected, especially since the juveniles of *L. sceleratus* can be misidentified for *Spicara smaris*, *Boops boops* and *Atherina hepsetus* (Katikou et al., 2009; Kalogirou, 2013) if fishermen are unfamiliar with the species. See also Q1.7b. The species is also proposed to be monitored through the Data Collection Reference Framework (DCRF) and the discards monitoring program of the GFCM (GFCM – UNEP/MAP, 2018).
2.8. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	very likely	high	During natural dispersal, organisms usually arrive and settle in suitable habitats or move on. Suitable habitats for the different life stages of *L. sceleratus* are

			widespread in the RA area. See Q1.9a, 1.15
2.9. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible)	rapidly	high	*L. sceleratus* was first detected in the Mediterranean Sea in 2003 (Filiz & Er, 2004 / Akyol et al., 2005) and in the RA area in 2004 (Cyprus: DFMR, 2006). Since then it has spread unaided throughout the Mediterranean Sea, so that it is considered one of the fastest expanding invasive species in the basin (Peristeraki et al., 2006, Coro et al., 2018). The Mediterranean populations appear to be tolerant of temperatures similar to those encountered at the low end of its thermal range, indicating potential adaptability to even cooler conditions. Further spread is expected into currently uninvaded areas as described in the Risk of Establishment section and population explosions may be triggered by one particularly warm summer.
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	very difficult	very high	There is a large consensus that naturally dispersing organisms are very difficult to contain (e.g. Carlton, 1996). This is particularly true for *L. sceleratus* due to the already widespread and abundant populations, the high fecundity, mobility and the long pelagic duration of the early life stages of the species.
2.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues and please provide quantitative data where possible).	rapidly	high	See Q2.9 and Q1.27 (Risk of Establishment) Current conditions: Mediterranean - Western Mediterranean Sea, Ionian Sea Iberian Shelf & Bay of Biscay, Sea of Marmara (reduced risk and rate of spread both because of less suitable climatic conditions but also due to the prevailing surface currents flowing towards the

			Mediterranean both in the Straits of Gibraltar and the Turkish Straits – unaided spread would most likely require active migration by adults)
2.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (please provide quantitative data where possible)	rapidly	medium	See Q1.28 (establishment under future conditions) For future climate change predictions, RCP 4.5 and 8.5 scenarios were considered. Future conditions: Mediterranean - Western Mediterranean Sea, Ionian Sea Iberian Shelf & Bay of Biscay, Sea of Marmara Future conditions will favour spread in areas that were previously of low suitability (like the North Adriatic and the Gulf of Lyon), but the model predicts little potential expansion of the suitable region into the Atlantic, the main limitation being low mean SST and possibly the upwellings along the Portuguese coast.

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 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)

QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**

Biodiversity and ecosystem impacts			
2.13. How important is 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?	moderate	low	Since it was first detected in the Mediterranean (Filiz & Er 2004; Akyol et al., 2005), *L. sceleratus* quickly underwent a population explosion in the Levantine Sea and now constitutes an important part of the local ichthyofauna, based on fisheries catches. Gülşahin & Soykan (2017) found that it constituted 7% of the fish biomass in Gökova Bay. The frequency of occurrence in trawl catches in Antalya Bay (2009-2010) was 56.48% at depths between 25 and 150 m with a Catch Per Unit trawling Area (CPUA) of up to 22 kg/km2 (Özbek et al., 2017). *L. sceleratus* is suspected to cause declines in native species of fish, crustaceans and cephalopods through predation. This is based on expert opinion, informed by the dietary preferences of the species, combined with its large size, voracity and increasing population densities, along with anecdotal reports from local fishermen (Kalogirou, 2013; Nader et al., 2012; Ünal et al., 2015). *L. sceleratus* is an opportunistic feeder with an ontogenetic shift in diet with increased body size from invertebrates (e.g. shrimps and crabs) and fish to molluscs, primarily cephalopods (Sabrah et al., 2006; Aydin, 2011; Kalogirou, 2013). Thus, fishermen in Gökova Bay (Turkey) have mentioned substantial decrease in *Mullus surmuletus* and *Octopus vulgaris* fisheries after *L. sceleratus* dominated the habitats and a collapse in the shrimp fishery of the region (Kizilkaya et al., 2014), where the stomach content of *L. sceleratus* comprises 85.2% of crustacea (Irmak, 2012). Similar complaints for reduced cephalopod densities, attributed to *L. sceleratus*, were expressed throughout the Turkish Levantine coast (Ünal et al., 2015) and in Egypt (FAO GFCM, 2013).

			However, there is no direct evidence of impact, partly due to the paucity of long-term, reliable fisheries data and background invertebrate data in many of the affected areas and partly because it is difficult to disentangle possible predation impacts of *L. sceleratus* from other factors that may be affecting native species population declines (Galanidi et al., 2018).
2.14. 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)?	moderate	low	In the RA area, the countries mostly affected so far by *L. sceleratus* are Greece and Cyprus. *L. sceleratus* persists dramatically in the Dodecanese and Crete areas of the Southern Aegean, where its abundance and biomass are continuously increasing. Studies conducted in 2009 revealed that *L. sceleratus* was present in three of four sets of trammel nets, resulting in 26% of total biomass (Corsini-Foka and Pancucci-Papadopoulou, 2010). This ratio has climbed to 63% in two of three sets of trammel nets, studied in November 2015, (M.Corsini-Foka, unpublished data). *L. sceleratus* was found to rank among the 10 most dominant fish species in terms of biomass in *Posidonia oceanica* habitats (Kalogirou et al., 2010) and among the ten most dominant species, both in terms of biomass and number of individuals, on sandy bottoms (Kalogirou et al., 2012). In Crete, the frequency of occurrence in fishers' catches was 30% in 2013 and had reached 57% in 2017 (N. Peristeraki, HCMR unpublished data). In 2017, fishers in Crete reported up to 800 kg of *L. sceleratus* per vessel per day of fishing (the link to this and all newspaper articles cited in this RA is provided in Annex VI, together with a brief summary where the article is not in English), and their catches appear to be

			dominated by ever larger individuals. Maximum reported CPUA in 2009 was 235kg/km2 (Koulouri et al., 2015). Suspected impacts on biodiversity are as described above (Q2.13), with lack of hard evidence hampering inference on cause/effect relationships. Catch per Unit Effort (CPUE) data from Cyprus DFMR Annual Reports for the years 2001 to 2013 (ANNEX XI) indicate population declines of the cephalopod species *Sepia officinalis*, *Loligo vulgaris* and especially *Octopus vulgaris* after 2006-2007, when *L. sceleratus* started becoming a dominant component of the Cypriot ichthyofauna but again, it is difficult to assign causality. Similar declines were observed in Greece, around the island of Kalymnos in the Dodecanese after 2009, according to unpublished landings data from the local Sea Fisheries Committee (Corsini-Foka & Emmanouil Perrakis, personal communication, January 2018).
2.15. 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?	major	low	*L. sceleratus* has already established populations in the eastern and central Mediterranean Sea and has been recorded in the western Mediterranean, where it is expected to establish within the RA area. There are growing concerns about its potential impacts on prey populations in these areas, especially cephalopod species. Based on its trophic level, lack of predators and low magnitude of fisheries removals (removals only occur as bycatch) it is considered likely that it can significantly affect marine food webs, exercising top-down control on prey species. Based on the currently available information, severe declines of cephalopod, crustacean and fish populations of prey species may be anticipated beyond the local scale but due to the poor level of documentation of the existing impacts, high

			uncertainty is associated with this assessment.
2.16. 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?	moderate	low	- Infralittoral muddy sand (EUNIS A5.24) - Listed as endangered natural habitat type in the Council of Bern Convention Resolution no. 4 (1996): Sublittoral soft seabeds (code 11.22) - Circalittoral fine sand (EUNIS A5.25) and all other EUNIS level 4 (A5.2, A5.3, A5.4) biotopes that fall under the 11.22 code - Sublittoral seagrass beds (EUNIS A5.53) – used as spawning habitats by *L. sceleratus* which can reduce their nursery value through predation – of which A5.535 *Posidonia oceanica* beds in the Mediterranean infralittoral zone, are considered vulnerable and A5.53 Seagrass beds on Atlantic infralittoral sand (non Macaronesian) are Critically Endangered (EU Red List of Habitats) Natura 2000 protected sites (georeferenced data available at https://www.eea.europa.eu/data-and-maps/data/natura-9) The current distribution of *L. sceleratus* overlaps with 38 Natura2000 sites that contain a marine area (see Annex X for the relevant map). Of these, 31 belong to Greece (29) and Cyprus (2), where the species is already invasive. 15 sites (11 in Greece) are fully marine, whereas the remaining 23 contain variable percentages of marine areas. The habitat components under protection within these sites are primarily the following: • Posidonia beds • Submerged or partially submerged sea caves • Sandbanks which are slightly covered by sea water all the time (includes Zostera beds)

			• Mudflats and sandflats not covered by seawater at low tide • Large shallow inlets and bays • Reefs • Estuaries & coastal lagoons (infralittoral/subtidal) Conservation value will be threatened primarily through predation and this will be more severe in habitats that are used as nursery grounds for fish and invertebrates, as already mentioned for Posidonia beds. There is no evidence to suggest that the structural complexity of the meadows will be impacted.
2.17. 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?	moderate	low	The habitats and protected sites mentioned in Q2.16 are expected to be impacted through predation also in the future. Additional protected sites in the Central and West Mediterranean are also at risk, according to the future establishment of *L. sceleratus* (Annexes VIII and X).
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	major	medium	The observed impact of *L. sceleratus* on ecosystem services is caused by changes introduced in the food-web (e.g. over-competing native species and decreasing populations of prey species). Furthermore, the species' toxicity makes it hazardous for human consumption which can negatively impact cultural values linked to recreational fishing (See 2.26). *L. sceleratus* has demonstrated impacts on food provisioning services, by interfering with commercial fisheries, and on cultural recreational services by affecting recreational fishing. More specifically, numerous studies document the increase in the frequency of occurrence and the percentage abundance and biomass of the species in fisheries catches (foregone catches are assumed as a result) (see Q 2.21-2.22 for details and references). Declines in wild stocks

			of cephalopods and commercial fish species (e.g. red mullets) have also been attributed to predation by *L. sceleratus* (Q 2.13). Recreational fishermen in Turkey (Iskenderun Bay) report that angling catches have severely declined or are impossible due to attacks from *L. sceleratus* on baited or unbaited angling lines (Arslantaş et al., 2017). Moreover, even though no information has been found on the issue, it is suspected that *L. sceleratus* may affect life-cycle maintenance (regulating services) provided by *P. oceanica* beds by decreasing their nursery value through predation on juvenile fishes (Kalogirou et al., 2010).
2.19. 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)?	major	medium	Within the RA area, impacts on food provisioning services (commercial fishing) and cultural services (recreational fishing) similar to those reported in Q2.18, are also evident in Cyprus (Levantine Sea) and Greece (Southern Aegean). There are also reports of attacks to spearfishers' catches, while spearfishing underwater (newspaper article, Annex VI), destroying the caught fish and inducing feelings of fear (Galanidi et al., 2018).
2.20. 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?	major	medium	The expanding distribution and increasing abundance and biomass of *L. sceleratus* in the RA area has the potential to inflict major impacts on ecosystem services (provisioning and cultural – see above) throughout the Mediterranean Sea.
Economic impacts			
2.21. 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	major	high	The economic costs / losses are linked to the impacts on commercial and recreational fisheries in terms of damage to fishing gear, increased demand for labour and predation on fisheries target species. *L. sceleratus* constitutes one of the most significant fisheries pests in the Eastern Mediterranean, where it

			has attained consistent and abundant populations. It damages fishing gear by attacking fish caught in nets and lines (Nader et al., 2012), thus causing both gear and labour losses but also catch losses. In some areas, many fishermen have even altered their fishing methods (gear, depths, time of the day, etc.) in order to avoid interaction with this species (Katsanevakis et al., 2009), while reinforcement of fishing lines using steel in another popular mitigation measure employed by fishermen (Kalogirou, 2013; Nader et al., 2012). Data on the effectiveness and costs of the above measures have not been reported. In contrast, gear and labour losses by artisanal fishermen in Turkey were investigated by Ünal et al. (2015) and Ünal & Göncüoğlu Bodur (2017) and were estimated to amount to a total [of 4,719 fishers have suffered a loss of] approximately EUR 2 million in 2011-2012 and >4.5 million EUR in 2013-2014, with economic losses more than doubling between 2011-2012 and 2013-2014. These calculations do not include foregone catches (either due to direct predation on fish caught in nets/lines or through predation impacts on prey populations of commercial importance) which are much more difficult to estimate but are nevertheless a valid and growing concern among small-scale fishermen in the Eastern Mediterranean (Panagopoulou et al., 2017).
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	major	medium	Financial estimates for economic losses in EU MS within the RA area are not available, however damages similar to Turkish fisheries (i.e. gear, labour and catch loss) have been extensively reported in the Dodecanese Islands and Crete (Greece) by Panagopoulou et al., (2017), Kalogirou (2013), Pancucci-Papadopoulou & Kalogirou (2013) and in Cyprus (DFMR, 2008; Katsanevakis et al., 2009). In a survey of Cretan small-

			scale fishermen (Panagopoulou et al., 2017), 19% of the respondents claimed that they suffered daily extensive damages caused by the invasive silver-cheeked toadfish *L. sceleratus*. Additionally, the frequency of occurrence in fishers' catches has almost doubled between 2013-2017 (see Q 2.14). In a recent local newspaper article (link in Annex VI), Cretan fishermen reported a catch of >800kg of *L. sceleratus* (≈200 large individuals weighing 4-6kg each), along with 10kg of red mullets *Mullus surmuletus* and 20kg of eaten/destroyed red mullets and damaged fishing nets that required 3 days of labour to mend. With respect to catch losses through predation on commercially important species, fishermen in Cyprus (EastMed 2010), Rhodes (Kalogirou, 2013) and Crete (Panagopoulou et al., 2017) have attributed the declines in cephalopod catches to the increasing populations of *L. sceleratus*. These claims are currently only tentatively substantiated by circumstantial evidence (see Q2.14) but urgently require further scrutiny based on additional data and modelling studies (Galanidi et al., 2018). A food-web model in Cyprus is currently under preparation (Michailidis et al., in prep.) Additional costs can also occur linked to negative effects on human health (e.g. treatment of poisoning) and possible diminishing of recreational values due to such risks (see Q2.26). Social and health costs of fatal incidents are very difficult to be monetised; relevant values are not available (e.g. life insurance compensation).
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area?	massive	medium	With *L. sceleratus* well established in the Aegean and with fast developing populations in the Ionian Sea and the Central Mediterranean (Jribi and Bradai 2012; Ben Souissi et al. 2014; Azzurro et al., 2016) as well as

*i.e. excluding costs of management			expanding its distribution in the Adriatic and Western Mediterranean (Sprem et al., 2014, Kara et al., 2015; Azzurro et al., 2018; Izguerdo-Munoz & Izguerdo-Gomez, 2014), it is expected to inflict financial damage on the fisheries of these regions as well. This will depend largely on the population densities the species reaches in the rest of the RA area. The north Aegean populations and Adriatic records indicate that the species is able to survive and possibly flourish even in the colder regions of the Mediterranean Sea and extend into Atlantic waters. This is also supported by the results of the habitat suitability model (this study, see Appendix). In these cooler regions, summer SST values close to a tentatively estimated thermal limit for reproduction (21.7 °C) can create conditions less favourable for prolific spawning but, considering the adaptability of *L. sceleratus*, the species may be able to overcome such limitations. Thus, higher uncertainty is attached to predictions in the current edges of its distribution. Based on the estimates for Turkish fisheries and the recent information from Crete indicating abandonment of commercial fishing due to unaffordable damages from *L. sceleratus* (see Q2.21 and Q 2.26 respectively), *L. sceleratus* has the potential to cause massive economic costs in the future in the RA area.
2.24. 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)?	major	medium	The gear and labour losses of Turkish fishermen presented in Ünal et al. (2015) and Ünal & Göncüoğlu Bodur (2017) can give an indication of the mitigation costs associated with fisheries impacts, which are currently shouldered primarily by the fishermen themselves, i.e. replacing damaged gear, which constitutes the largest proportion of the economic costs estimated in these two studies. A bounty program was first implemented in Cyprus in

			2009-2010 in order to make possible the collection of sufficient specimens of *L. sceleratus* for the study of the population characteristics of the species (Michailidis, 2011 in Greek). Since then, population control campaigns with intensive targeted fishery of the breeding population of the species in the summer months have taken place in Cyprus in 2012-2016. The amount paid to beneficiaries was €3/kg and it amounted to €600K in 5 years through a management plan partly covered by European fisheries funds [€102480 (34160 kg) in 2012, €43.800 in 2013, €164.940 (54.980 kg) in 2014, €249.465 (83.155 kg) in 2015, €41.235 (13.745 kg) in 2016] (DFMR Cyprus 2011, 2012, 2013, 2014, 2015, 2016). Costs associated with awareness campaigns specific to *L. sceleratus* currently in place in a number of EU Member States (Greece, Cyprus, Italy, Malta) and Early Detection/Rapid response networks for marine invasive species in general should also be taken into account. Monetary values for such activities were not found.
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	major	medium	Managing *L. sceleratus* will require studies of its populations, with particular emphasis on its reproductive fields and the effectiveness of targeted actions. Intensive targeted fishery with a bounty system can provide some financial compensation to fishermen but, based on the Cyprus experience, has not proven to be successful. The Hellenic Centre for Marine Research (HCMR) is considering the development of pheromone traps as an alternative method to collect mature reproductive individuals but this could incur considerable costs (Stewart & Sorensen, 2015). Due to its high toxicity, associated marketing regulations in EU and import regulations in Asian countries consuming fugu, a commercial fishery of *L. sceleratus* for consumption is not a management option.

			However, collected specimens through a bounty system could be used to research the feasibility of TTX extraction for pharmaceutical uses.
Social and human health impacts			
2.26. 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).	major	medium	*L. sceleratus* is a toxic species with severe risks to human health. Its aggressive and voracious behaviour negatively impacts wellbeing related values by limiting fishing related recreation. In addition, broader social impacts can be caused due to negative impacts on local livelihood and abandonment of fishing by small-scale fishermen, resulting in societal changes (e.g. unemployment). *L. sceleratus* is capable of accumulating tetrodotoxin (TTX) in its tissues, particularly the liver, gonads, intestine and skin, but also in muscle tissue (Katikou et al., 2009). TTX is a potentially lethal neurotoxin (strong sodium channel inhibitor) that causes a range of symptoms, from mild paraesthesia and nausea to full paralysis, hypotension, and death from respiratory failure (Field, 1998). Symptoms typically begin within 30 minutes after ingestion and the mortality rate in humans is 60% (refs in Field, 1998). Because pufferfishes are considered a delicacy in some Asian countries, pufferfish intoxication incidents have been reported in Asian coastal areas (Islam et al, 2011 in Guardone et al., 2018). The marketing of *L. sceleratus* is prohibited in the EU (EC No 1021/2008) and many non-EU Mediterranean countries have introduced their own restrictions for the fishing, landing and selling of the species, but it is nevertheless still consumed in some countries after the removal of the head and the internal organs (Aydın, 2011; Beköz et al., 2013). Fatalities from *L. sceleratus* consumption have been

			reported from Suez City and Alexandria in Egypt (Zaki, 2004; Elshama et al., 2011; Halim & Rizkalla, 2011), Lebanon (Nader et al., 2012), Syria (newspaper articles in the local press) and Libya (Shakman, pers.comm.), while numerous cases of severe poisoning are also documented in the aforementioned countries (Chamandi et al., 2009; Elshama et al., 2011) and also in Israel (Bentur et al., 2008), Tunisia (Ben Souissi et al., 2014), Cyprus (local press) and Greece (local press – links to the newspaper articles with a brief translated summary are provided in Annex VI). In Turkey, symptoms of intoxication have been reported from small-scale fishermen, some of who still land and consume the fish themselves (Ünal et al., 2015; Ünal & Göncüoğlu Bodur, 2017), or even illegally sell it to unsuspecting customers (Beköz et al., 2013). [In Italy, 10 people were intoxicated in Rome and Jesolo (Venice) in 1977, after the consumption of toxic Tetraodontidae from Taiwan, mixed with batches of monkfish (*Lophius piscatorius*) (Pocchiari, 1977 in Guardone et al., 2018), however cases of intoxication due to consumption of invasive individuals in the wild have not been reported to date.] Apart from direct impacts on human health, *L. sceleratus* in the invaded areas is also impacting recreational activities, primarily recreational fishing, by attacking fish caught in anglers' lines or dominating their catches (Arslanta et al., 2017) or by attacking and inducing feelings of fear in spearfishers (newspaper article in Greek). However, the most severe social impact may well be the abandonment of fishing as a livelihood activity by small-scale fishermen locally. In 2018, a "large number of small-scale fishermen from Crete have requested the withdrawal of their fishing vessels, as they cannot

			withstand the financial damages caused by *L. sceleratus*" (Nota Peristeraki, HCMR, pers. comm.).
2.27. 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.	major	medium	Despite the well-known toxicity of the species, legislation prohibiting its landing and consumption and numerous awareness campaigns in Eastern Mediterranean countries (Ben Souissi et al., 2014 and references therein), the information has apparently not reached all those potentially affected and unsuspecting consumers still remain vulnerable, at least in the Eastern Mediterranean (Beköz et al., 2013; Ben Souissi et al., 2014; Ünal et al., 2015; Ünal and Göncüoğlu Bodur, 2017), including tourists and maritime professionals. With the expansion and predicted establishment of the species into new regions of the RA area, more people will be at risk. Additionally, the growing (in abundance and age/size structure) populations of *L. sceleratus* pose a strong and valid threat to small-scale fisheries in the invaded areas, with some permanent change in the activity locally already evident and concerns being expressed over a wider area.
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	minor	low	To date, there has only been one published study on *L. sceleratus* parasitism in the Mediterranean invaded range (Bakopoulos et al., 2017) and scattered information for the native range. Bakopoulos et al. (2017) found two nematode genera and one Gnathiid isopod parasite in *L. sceleratus* specimens from the Aegean Sea. All three parasites are indigenous with low host specificity in the Mediterranean and low mean intensity of infection in *L. sceleratus*. The authors of the study concluded that *L. sceleratus* provides an additional niche for the success and increase of local populations of these parasites, but its invasion success in unlikely to be affected by them.

			In its native range, *L. sceleratus* is reported to act as host to digenean parasites of the genus *Zoogonides* that are also common in the north-eastern Atlantic, using predominantly flatfish as their definitive host (Bray and Justine, 2014), so it can potentially act as an agent of spread for these trematode parasites as well.
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	NA		
2.30. 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?	major (environment) massive (socio-economy)	medium	*L. sceleratus* is generally considered to have few natural enemies, both in the native and the invaded range (East Med, 2010) owing to successful predation avoidance mechanisms (see Q1.18). An incidence of *L. sceleratus* juvenile predation by the common dolphinfish *Coryphaena hippurus* off Crete was recently reported by Kleitou et al. (2018) but the scope for natural control of *L. sceleratus* due to *C. hippurus* predation is still unclear. Other large, coastal or pelagic predators, which are present in the Mediterranean, are known to consume pufferfish species in the native range (Mohamed et al., 2013) but the potential for population control of *L. sceleratus* is unknown (see also Q1.18). Low prevalence of the ectoparasitic isopod Gnathia spp. (Bakopoulos et al., 2017) is not expected to alter the magnitude of impacts of *L. sceleratus*. With respect to digenean parasitism, the presence of adult, reproducing digeneans in the definitive host forces the host to intensify their search for food, resulting in decreased fitness and an increased risk of being eaten (Bartoli and Boudouresque, 2007); thus, on one hand it may lead to increased likelihood of predation of *L. sceleratus* but on the other hand it has

			the potential to increase the level of predation by *L. sceleratus* individuals, which is already assumed to be the most severe ecological impact of this species.

ANNEXES

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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**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[10]	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 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.

[10] Not to be confused with „no impact".

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.*. 2017)

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[11]	**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.*

[11] 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.

	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** 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*

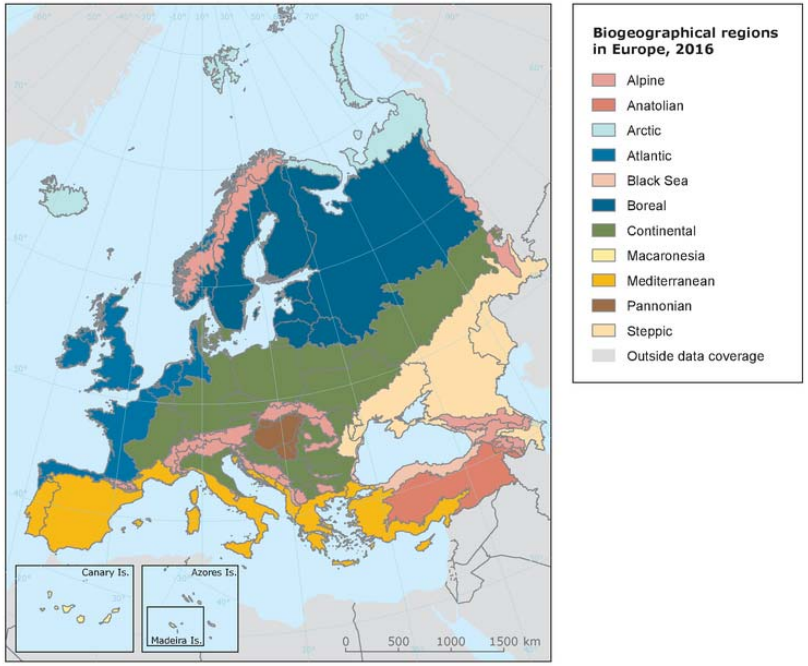
			composition etc.) that make it attractive for recreation, wild life watching etc.
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living 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.*

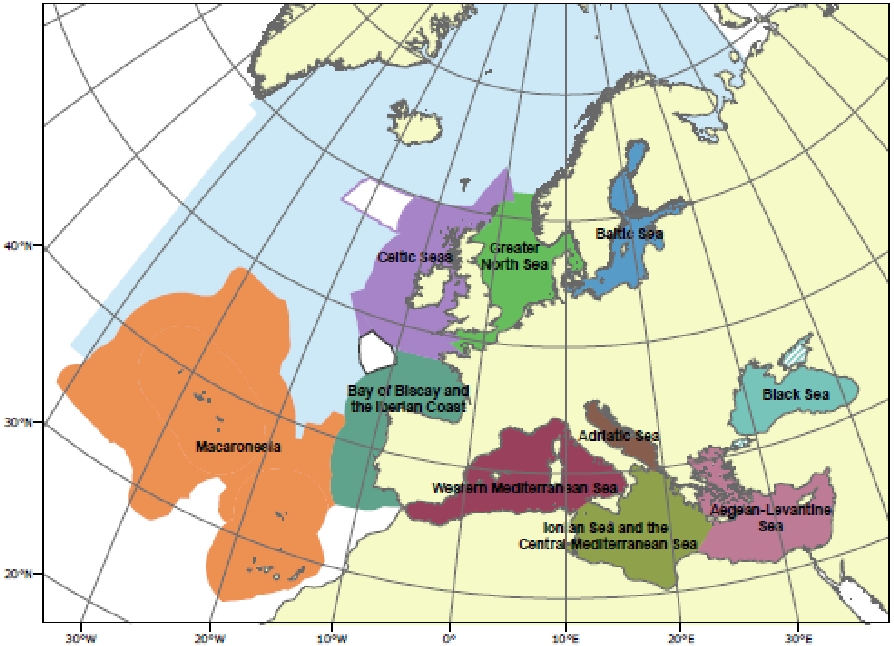
ANNEX V EU Biogeographic Regions and MSFD Subregions

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 Socio-economic impacts of L. sceleratus in the invaded range (adapted from Galanidi et al., 2018).

Constituent of human well-being	Activity	Citation from text	Full Reference	Location	Comments
health	multiple activities	Thirteen patients aged 26–70 years were admitted (to the hospital between 2005-2008) after consuming L. sceleratus. Signs of toxicity appeared within 1 h. The main manifestations included vomiting, diarrhea, headache, paraesthesias, slurred speech, muscle weakness, dyspnea, hypertension, tachycardia, respiratory arrest, seizures and coma. Treatment was supportive, including mechanical ventilation (two patients). Patients recovered within 4 days. The most severely poisoned patient (a 33 year old healthy male) ate the entire liver of the fish. His symptoms began within 10 min, during the meal, and rapidly progressed. He suffered from whole body paraesthesias, vomiting, dyspnea, and hypertension (240/120 mmHg), lost consciousness, became cyanotic and required mechanical ventilation for 24 h. Another severely poisoned patient ate almost a whole fish liver.	Bentur, Y., Ashkar, J., Lurie, Y., Levy, Y., Azzam, Z. S., Litmanovich, M., et al. (2008). Lessepsian migration and tetrodotoxin poisoning due to *Lagocephalus sceleratus* in the eastern Mediterranean. Toxicon 52, 964–968. doi:10.1016/j.toxicon.2008.10.001.	Israel (Haifa to Ashkelon, along the coast)	

health	multiple activities	A 68 year-old woman, with hypertension and diabetes, was brought to the Emergency Department of the Hopital Universitaire de Notre Dame De Secours (Lebanon), in January 2008 complaining of proximal limb weakness and dyspnea. Four hours prior to her arrival, the patient had eaten a half-cooked fish liver (later identified as coming from L. sceleratus). Three hours and thirty minutes later, she started feeling a tingling sensation in the perioral region and in the tip of her fingers associated with blurred vision, head heaviness, nausea and one episode of vomiting. Ten minutes later, she lost her ability to hold her head up and had developed weakness of her upper and lower extremities. This was accompanied by mild abdominal distention and urinary urgency. The patient then developed quadriplegia, hypophonia and dysarthria. She complained of dyspnea, ophtalmoplegia and had an absent gag reflex. Subsequently, the patient underwent endotracheal intubation. (The woman recovered after treatment.)	Chamandi, S. C., Kallab, K., Mattar, H., and Nader, E. (2009). Human Poisoning after ingestion of puffer fish caught from Mediterranean sea. Middle East J. Anaesthesiol. 20, 285–288.	Lebanon	
health	multiple activities	Despite the awareness campaign, a serious case of intoxication by silver-cheeked toadfish {another common name for the pufferfish L.sceleratus} was registered in August 12, 2013 in Gafsa, an inland town located about 110 Km (airline) West from the (Tunisian) coastline. This incident, immediately reported by national television and newspapers, was attributed to the commercialization of L. sceleratus in the internal areas of the country, were no specific actions were carried out to inform people about the risks	Ben Souissi, J., Rifi, M., Ghanem, R., Ghozzi, L., Boughedir, W., and Azzurro, E. (2014). Lagocephalus sceleratus (Gmelin , 1789) expands through the African coasts towards the Western Mediterranean Sea : a call for awareness. Manag. Biol. Invasions 5, 357–362.	Tunisia, Gafsa	

		posed by this species.			
health	multiple activities	The most severe case of adverse impact on human health is that of the puffer fish, Lagocephalus sceleratus. Although sold beheaded and eviscerated, it soon proved to be a serious hazard to consumers, causing paralysis of the mouth and limbs. Four lethal cases occurred in Alexandria following consumption of L.sceleratus due to tetrodotoxin.	Halim, Y., and Rizkalla, S. (2011). Aliens in egyptian mediterranean waters. A check-list of Erythrean fish with new records. Mediterr. Mar. Sci. 12, 479–490.	Egypt, Alexandria	
health	multiple activities	In Egypt, many physicians observed sporadic cases of food poisoning after eating this specific type of fish. In the last ten years, the rate of these cases has progressive increased...toxicity of puffer fish is now a common form of poisoning throughout Egyptian coastal cities such as Suez city. In Suez city, the majority of people eat puffer fish without developing any toxic manifestations, but some of them complain of different toxic signs and symptoms without any residual affection and others die .	Elshama, S. S., Zaki, M. A., and Metwally, M. E. (2011). Factors affecting the clinical picture & prognosis of puffer fish poisoning in Suez city during year of 2008. Ain Shams J. Forensic Med. Clin. Toxicol. XVI, 99–109.	Egypt, Suez city	impact from the native range

health	multiple activities	TTX (tetrodotoxin) is not destroyed by heat while cooking the fish and intoxication cases are mainly due to lack of awareness among consumers, misidentification of species or due to erroneous traditional conception of detoxification methods.	Nader, M., Indary, S., and Boustany, L. (2012). The Puffer Fish Lagocephalus Sceleratus (Gmelin, 1789) in the Eastern Mediterranean. Gcp/Int/041/Ec – Gre – Ita/Td-10, 1–33.	Eastern Med	
health	multiple activities	Nine patients with history of puffer fish ingestion transferred to General Suez Hospital. According to clinical grading system for tetrodotoxin toxicity, three patients had grade 1 and another three had grade 2 but, three fatal cases had grade 3 and their condition worsened and became grade 4. Fatal cases ate gonads and head of fish and the cause of death was respiratory failure. Grade 1 is characterized by perioral numbness and paraesthesia while grade 2 shows numbness of tongue, face and other areas, incoordination and slurred speech. Grade 3 is manifested by flaccid paralysis, dyspnoea and aphonia . Grade 4 is respiratory failure and coma.	Elshama, S. S., Zaki, M. A., and Metwally, M. E. (2011). Factors affecting the clinical picture & prognosis of puffer fish poisoning in Suez city during year of 2008. Ain Shams J. Forensic Med. Clin. Toxicol. XVI, 99–109.	Egypt, Suez city	impact from the native range, not scored
health	multiple activities	This species is also consumed in Lebanon by some fishers and a small number of consumers ignorant of the health threats it poses where several cases of unofficial intoxication have been reported in that country after eating L. sceleratus. The only official record was in 2008 when a 68 year old woman complaining of limb weakness and dyspnea was brought to a hospital in Beirut. The family revealed after questioning that she had eaten a half-cooked liver of L. sceleratus (Chamandi et al., 2009, see record a few rows above - this is not scored here). Even though in that particular case the woman	Nader, M., Indary, S., and Boustany, L. (2012). The Puffer Fish Lagocephalus Sceleratus (Gmelin, 1789) in the Eastern Mediterranean. Gcp/Int/041/Ec – Gre – Ita/Td-10, 1–33.	Lebanon	

		survived, the local media records seven cases of death (this is the scored record) in the past few years in Lebanon due to consumption of puffer fishes. As a result, the Lebanese authorities banned in 2011 the fishing, selling and consuming of all puffer fishes including L. sceleratus.			
health	multiple activities	In this study (conducted between 2011-2012), 29% of the (261 interviewed) fishers admitted they have consumed Lagocephalus sceleratus at least once and consequently 18.5% of them have reported health issues following consumption	Ünal, V., Göncüoğlu, H., Durgun, D., Tosunoğlu, Z., Deval, M. C., and Turan, C. (2015). Silver-cheeked toadfish, Lagocephalus sceleratus (Actinopterygii, Tetraodontiformes: Tetraodontidae), causes a substantial economic losses in the Turkish Mediterranean coast : A call for decision makers. Acta Ichthyol. Piscat. 45, 231–237. doi:10.3750/AIP2015.45.3.02.	Turkey, all along the south coast	

health	multiple activities	In 2013-2014, 38% of the (215 interviewed) fishers reported that they consumed pufferfish, and 11% of those who consumed it stated that they experienced the symptoms of intoxication. An increase in the consumption of pufferfish (compared with the 2011-2012 study, see above) despite its being poisonous shows that studies on raising the awareness in this regard have not been effective enough.	Ünal, V., and Göncüoğlu Bodur, H. (2017). The socio-economic impacts of the silver-cheeked toadfish on small-scale fishers: A comparative study from the Turkish coast. Ege J. Fish. Aquat. Sci. 34, 119–127. doi:10.12714/egejfas.2017.34.2.01.	Turkey, all along the south coast	
health	multiple activities	All fishermen (25 interviewed anglers) had previously been notified that puffer fish were poisonous but did not believe this was truly the case, admitting that they had all caught and regularly sold puffer fish; five fishermen and fish dealers claimed to have sold puffer fish to local hotels. All 37 stall workers and/or owners of the 11 open market fish stands appeared anxious on questioning and denied ever buying puffer fish from anglers or dealers, or selling puffer fish to the public, despite puffer fish being physically present for sale on some stands. Seventy-eight per cent of (100 interviewed) customers in the same areas as the open market fish stands had never heard of puffer fish. Of the 22 customers who had heard of puffer fish, only five were aware of the poisonous nature of puffer fish. Two-thirds of the customers bought seafood according to the seller's advice. Three-quarters of the customers could not visually distinguish puffer fish from other fish.	Beköz, A. B., Beköz, S., Yilmaz, E., Tüzün, S., and Beköz, U. (2013). Consequences of the increasing prevalence of the poisonous Lagocephalus sceleratus in southern Turkey. Emerg. Med. J. 30, 954–5. doi:10.1136/emermed-2011-200407.	Turkey, Antalya	

health	multiple activities	Ukrainian tanker TAMANSKIY requested immediate assistance on Dec 23 13, reporting mass food poisoning on board. Vessel was off Crete Island, Mediterranean sea, en route to Damietta, Egypt. Greek Coast Guard patrol boat was dispatched to pick up ill seamen. Boat took on board five crew members and delivered them to Ierapetra, Crete. They've been transferred to local hospital.	https://www.fleetmon.com/maritime-news/2013/2876/mass-poisoning-board-ukrainian-tanker-tamanskiy-me/	Greece, Crete	
health	multiple activities	Ο Ουκρανός καπετάνιος, ο οποίος είχε πέσει σε κώμα όταν έφαγε λαγοκέφαλο μαζί με άλλους τέσσερις συναδέλφους του στα ανοικτά της Ιεράπετρας, φαίνεται πως γλυτώνει το θάνατο. Ενώ είχε πέσει σε κώμα και η κατάσταση του ήταν εξαιρετικά κρίσιμη, ο Ουκρανός άρχισε να αισθάνεται καλύτερα, όπως αναφέρει το cteteplus.gr. Οι πέντε ναυτικοί είχαν καταναλώσει λαγοκέφαλα που είχαν ψαρέψει ενώ ταξίδευαν νότια της Ιεράπετρας, με τον καπετάνιο να είναι χειρότερα, αφού είχε φάει και τα εντόσθια του ψαριού. Μόλις τελείωσαν το γεύμα τους όμως, ένιωσαν έντονη αδιαθεσία, με αποτέλεσμα να στηθεί μια μεγάλη επιχείρηση για τη μεταφορά τους στο νοσοκομείο της Ιεράπετρας.	http://www.iefimerida.gr/news/136604/n%CE%B1%CF%85%CF%84%CE%B9%CE%BA%CE%BF%CE%AF-%CE%AD%CF%86%CE%B1%CE%B3%CE%B1%CE%BD-%CE%B1%CF%85%CF%84%CF%8C-%CF%84%CE%BF-%CF%88%CE%AC%CF%81%CE%B9-%CE%BA%CE%B1%CE%B9-%CE%AD%CF%80%CE%B5%CF%83%CE%B1%CE%BD-%CF%83%CE%B5-%CE%BA%CF%8E%CE%BC%CE%B1-%CF%83%CF%84%CE%B7%CE%BD-%CE%B9%CE%B5%CF%81%CE%AC%CF%80%CE%B5%CF%84%CF%81%CE%B1-%CE%BA%CF%81%CE%AE%CF%84%CE%B7%CF%82-%CF%80%CF%81%CE%BF%CF%83%CE%BF%CF%87%CE%AE-	Greece, Crete	same incident, additional information in Greek from local press

			%CF%83%CF%84%CE%BF%CE%BD-%CE%BB%CE%B1%CE%B3%CE%BF%CE%BA		
health	multiple activities	Local newspaper article reporting on the severe poisoning of two Russian tourists who consumed pufferfish that one of them fished in Cyprus. The 21-year old man entered a coma, whereas his 65-year old mother suffered a cardiac arrest and was successfully resuscitated. Both were intubated and admitted to the ICU but sucessfully recovered.	http://agonaskritis.gr/%CE%BA%CE%B9%CE%BD%CE%B4%CF%8D%CE%BD%CE%B5%CF%85%CE%BF%CF%85%CE%BD-%CE%BC%CE%B5-%CE%B8%CE%AC%CE%BD%CE%B1%CF%84%CE%BF-%CE%B1%CF%80%CF%8C-%CE%BA%CE%B1%CF%84%CE%B1%CE%BD%CE%AC%CE%BB%CF%89%CF%83%CE%B7/	Cyprus	newspaper article in Greek
health	multiple activities	Local newspaper article reporting on two deaths in Lattakia resulting from L. sceleratus consumption and numerous other cases of poisoning and deaths (8-10) around Syria. Article published on 04/11/2016	https://syriaalyom.com/index/%D8%A3%D8%B3%D9%85%D8%A7%D9%83-%D8%B3%D8%A7%D9%85%D8%A9-%D8%AA%D9%81%D8%AA%D9%83-%D8%A8%D8%A7%D9%84%D9%85%D9%88%D8%A7%D8%B7%D9%86%D9%8A%D9%86-	Syria, Lattakia	newspaper article in Arabic

			%D9%81%D9%8A-%D8%A7%D9%84%D9%84%D8%A7%D8%B0%D9%82%D9%8A/		
health	multiple activities	Local newspaper article reporting the death of two people and the poisoning of another three from the same family resulting from L. sceleratus consumption (raw flesh). Article published on 08/05/2014	http://emediatc.com/index.php?page=Details&category_id=10&id=8211	Syria, Tartous	newspaper article in Arabic
health	multiple activities	Local newspaper article reporting the death of 13 people resulting from L. sceleratus consumption in Lattakia, Syria in the past year alone. The article was published on 12/11/2017 and the information comes from the director of the Lattakia National Hospital.	http://www.sana.sy/?p=658645	Syria, Lattakia	newspaper article in Arabic
health	multiple activities	Local newspaper article reporting the death of a 4-year old Palestinian girl and the poisoning of 4 other members of her family after consuming L. sceleratus that was purchased from a street vendor. The family lived in a refugee camp in Sidon.	http://saidacity.net/mobile/_common.php?cache_time=0&news_id=14542	Lebanon	newspaper article in Arabic
Basic material assets, adequate hivelihood	commercial fishing	The voracious silver-cheeked toadfish (Lagocephalus sceleratus) forced longliners to use steel instead of nylon cables	Edelist, D., Scheinin, A., Sonin, O., Shapiro, J., Salameh, P., Rilov, G., et al. (2013). Israel: Reconstructed estimates of total fisheries removals in the Mediterranean, 1950-2010.	Israel	

Basic material assets, adequate hivelihood	commercial fishing	L.sceleratus often damages both the fishing gear and the catch of the fishermen with its powerful jaws	DFMR, 2008. Annual report on the Cyprus fisheries for the year 2008. Department of Fisheries and Marine Research. Ministry of Agriculture, Natural Resources and Environment, Nicosia, Cyprus	Cyprus	
Basic material assets, adequate hivelihood	commercial fishing	In some areas, many fishermen have even altered their fishing methods (gear, depths, time of the day, etc.) in order to avoid interaction with this species	Katsanevakis, S., Tsiamis, K., Ioannou, G., Michailidis, N., and Zenetos, A. (2009). Inventory of alien marine species of Cyprus (2009). Mediterr. Mar. Sci. 10, 109–133. doi:10.12681/mms.113.	Cyprus	personal observatio n of Ioannou & Michailidis
Basic material assets, adequate hivelihood	commercial fishing	the landings of the species reached around 4% of the total landings of the Cyprus inshore fisheries in 2009 and 2010	EastMed (2010). REPORT OF THE TECHNICAL MEETING ON THE LESSEPSIAN MIGRATION AND ITS IMPACT ON EASTERN MEDITERRANEAN FISHERY.	Cyprus	unpublish ed statistics of the Departme nt of Fisheries and Marine Research of Cyprus
Basic material assets, adequate hivelihood	commercial fishing	According to DFMR unpublished data and reports from the artisanal fishermen, there seems to be an effect of the increasing L. sceleratus population, at least since 2006, on the cephalopod populations in Cyprus (Figure 22 showing declines in annual landings of Octopus vulgaris, Sepia officinalis and Loligo vulgaris in Cyprus since 2006).	EastMed (2010). REPORT OF THE TECHNICAL MEETING ON THE LESSEPSIAN MIGRATION AND ITS IMPACT ON EASTERN MEDITERRANEAN FISHERY.	Cyprus	

Basic material assets, adequate hivelihood	commercial fishing	The relatively high percentage of stomachs including both fish and pieces of net {this study} could suggest that L. sceleratus often preys on fast swimming fish when they are already entangled in nets... items most probably used as bait (animal flesh and bones, potatoes etc.), as well as pieces of fishing net (8.4%) and hooks (0.2%) were also found	EastMed (2010). REPORT OF THE TECHNICAL MEETING ON THE LESSEPSIAN MIGRATION AND ITS IMPACT ON EASTERN MEDITERRANEAN FISHERY.	Cyprus	
Basic material assets, adequate hivelihood	commercial fishing	Lebanese fishers are complaining from the damage caused to their gears and their catch. Some have already started adding a metallic wire at the end of their fishing lines to prevent the fish from biting through them therefore reducing the loss of hooks and weights.	Nader, M., Indary, S., and Boustany, L. (2012). The Puffer Fish Lagocephalus Sceleratus (Gmelin, 1789) in the Eastern Mediterranean. Gcp/Int/041/Ec – Gre – Ita/Td-10, 1–33.	Lebanon	Reviw article, the particular citation refers to Lebanon
Basic material assets, adequate hivelihood	commercial fishing	When it comes to pot users {among Lebanese fishermen}, complaints reveal that this species either eats the catch or keeps fish away from entering the pots.	Nader, M., Indary, S., and Boustany, L. (2012). The Puffer Fish Lagocephalus Sceleratus (Gmelin, 1789) in the Eastern Mediterranean. Gcp/Int/041/Ec – Gre – Ita/Td-10, 1–33.	Lebanon	Reviw article, the particular citation refers to Lebanon
Basic material assets, adequate hivelihood	commercial fishing	L. sceleratus is considered a major nuisance by fishers since it damages fishing gear by attacking fish caught in nets and lines, along with reducing local stocks of squids and octopus through predation. This species can easily cut lines and nets using its strong teeth. All of the above is affecting the well-being of the fishing community by increasing the time spent fishing, the mending and replacing of damaged gears and cleaning nets from puffer fishes and their remains.	Nader, M., Indary, S., and Boustany, L. (2012). The Puffer Fish Lagocephalus Sceleratus (Gmelin, 1789) in the Eastern Mediterranean. Gcp/Int/041/Ec – Gre – Ita/Td-10, 1–33.		Synthesis from review article

Basic material assets, adequate hivelihood	commercial fishing	fishing nets {in L.sceleratus stomachs} occurred at a relatively high frequency of 12.6%, which shows that this invasive species is a voracious predator attacking fish captured in nets and is capable of ripping and ingesting them	Boustany, L., Indary, S. E. L., and Nader, M. (2015). Biological characteristics of the Lessepsian pufferfish Lagocephalus sceleratus (Gmelin , 1789) off Lebanon. Cah. Biol. Mar. 56, 137–142.	Lebanon	
Basic material assets, adequate hivelihood	commercial fishing	Face-to-face interviews were completed with a total of 261 fishers from Izmir in the Middle Aegean region to Hatay in the Eastern Mediterranean region, to determine the problems arising from the presence of silver-cheeked toadfi sh species in the ecosystem and the resulting associated economic losses for a 1-year from 1 January to 30 December 2011. 91% of the fishers interviewed considered L. sceleratus a major problem for their fishing activity and the responses on adverse effects on the capture efficiency were also similarly high (89%). 78% of fisher's fishing gear was damaged by Lagocephalus sceleratus with calculated related losses 1300 TRY per year, per fisherman;	Ünal, V., Göncüoğlu, H., Durgun, D., Tosunoğlu, Z., Deval, M. C., and Turan, C. (2015). Silver-cheeked toadfish, Lagocephalus sceleratus (Actinopterygii, Tetraodontiformes: Tetraodontidae), causes a substantial economic losses in the Turkish Mediterranean coast : A call for decision makers. Acta Ichthyol. Piscat. 45, 231–237. doi:10.3750/AIP2015.45.3.02.	Turkey, all along the south coast	
Basic material assets, adequate hivelihood	commercial fishing	Cretan fishermen reported a catch of >800kg of L. sceleratus (≈200 large individuals weighing 4-6kg each), along with 10kg of red mullets Mullus surmuletus and 20kg of eaten/destroyed red mullets and damaged fishing nets that required 3 days of labour from 5 people to mend.	https://www.newsit.gr/topikes-eidhseis/kriti-sikosan-ta-dixtya-tous-kai-eidan-800-kila-lagokefalous-katasparaksan-20-kila-mparmpounia/2450237/	Greece, Crete	newspaper article in Greek

Basic material assets, adequate hivelihood	commercial fishing	The research is based on a dataset collected between January 1st, 2013 and January 1st, 2014 using face-to-face interviews with 215 fishers from 7 coastal cities from İzmir-Çesme in the Middle Aegean region to Hatay-Samandağ in the Syrian border. Almost all of the fishers (97%) stated that this species damages the fish entangled in their nets. Therefore, it causes financial losses for the fishers by reducing fishing yield and value. The study revealed that 78% of fisher's fishing gear {longlines and gillnets} was damaged by Lagocephalus sceleratus; The fishing gear loss per vessel in 2013-2014 is 2,554 Turkish Lira/year and labor loss for longline is 64.7 TL/year. The damage caused by pufferfish to the fish entangled in the fishing gear could not be monetized. The monetary loss in small-scale fishing doubled within two years (compared with the 2011-2012 study, see above).	Ünal, V., and Göncüoğlu Bodur, H. (2017). The socio-economic impacts of the silver-cheeked toadfish on small-scale fishers: A comparative study from the Turkish coast. Ege J. Fish. Aquat. Sci. 34, 119–127. doi:10.12714/egejfas.2017.34.2.01.	Turkey, all along the south coast	
Basic material fassets, adequate hivelihood	commercial fishing	52 long-line hooks were found in 33 {L.sceleratus} stomachs, thus confirming the pest status of L. scleratus for commercial long-line fishermen	Kalogirou, S. (2013). Ecological characteristics of the invasive pufferfish Lagocephalus sceleratus (Gmelin, 1789) in Rhodes, Eastern Mediterranean Sea. A case study. Mediterr. Mar. Sci. 14, 251–260. doi:10.12681/mms.364.	Greece, Rhodes island	
Basic material assets, adequate hivelihood	commercial fishing	Adaptation of long- and handline fisheries included fishing in deeper areas (> 60 m), where L. sceleratus was considered to be absent, and reinforcement of fishing lines using steel	Kalogirou, S. (2013). Ecological characteristics of the invasive pufferfish Lagocephalus sceleratus (Gmelin, 1789) in Rhodes, Eastern Mediterranean Sea. A case study. Mediterr.	Greece, Rhodes island	

			Mar. Sci. 14, 251–260. doi:10.12681/mms.364.		
social and cultural relations, recreation	recreational fishing	A hobby fishing attempt in Fethiye Bay resulted with three broken fishing lines, ten missing hooks and one L.sceleratus caught (slightly over 1 kg), just within five minutes	Bilecenoglu, M. (2010). “Alien marine fishes of Turkey – an updated review,” in Fish Invasions of the Mediterranean Sea: Change and Renewal, eds. D. Golani and A. Appelbaum-Golani (Pensoft Publishers, Sofia-Moscow), 189–217.	Turkey, Fethiye Bay	
social and cultural relations, recreation	recreational fishing	This study was performed in order to determine how the increased pufferfish population has affected the region's angling. Data was obtained by conducting a survey with people (355 face-to-face) in recreational fishing activities from Iskenderun Bay. Pufferfish species are one fo the most caught species in the angling and longline fishery in the region. Although the pufferfish is considered a problem because of the damage it inflicts on the fishing gear, anglers are not too bothered by this situation; when they see the pufferfish caught, they prefer to cut the fishing line instead of landing the fish. However, they reported that it is not possible to catch an angling in the density of the pufferfish in this region because pufferfish species are always attacking regardless of whether it is bait or without bait.	Arslantaş E., Demirci, S., Demirci, A. (2017). Negative effects of pufferfish on the recreational fishery in Iskenderun Bay. International Symposium on Pufferfish, 13-14 October 2017, Bodrum Turkey.	Turkey, Iskenderun Bay	abstract only

social and cultural relations, recreation	recreational fishing	Reports the encounter of a recreational spear-fisher with approximately 25 pufferfish, while he was spear-fishing 500m from the coast. The pufferfish followed him while he was swimming towards the shore and managed to get all the fish he had caught and was carrying on him, causing strong feelings of fear.	http://www.neakriti.gr/?page=newsdetail&DocID=1330837	Greece, Crete	newspaper article in Greek
safety, secure resource access	recreational activities (going to the beach)	Reports the encounter of an 8 year old playing in shallow waters with 4 large pufferfish that approached her. No injuries were reported but the child and her family communicated feelings of fear	http://www.neakriti.gr/?page=newsdetail&DocID=1330837	Greece, Crete	newspaper article in Greek

ANNEX VII Clinical symptoms and prognosis of *Lagocephalus sceleratus* intoxication

Table 2
Distribution of the clinical manifestations of patients consuming *Lagocephalus sceleratus*

Clinical manifestations	Number of patients
Paraesthesias	10
Circumoral	8
Oral (e.g., lingual)	2
Limbs	8
Face	2
Entire body	1
Slurred speech	5
Muscle weakness[a]	6
Dyspnea	3
Dizziness	3
Ataxia	4
Headache (frontal, severe)	1
Absence of patellar reflexes	1
Abnormal cerebellar tests	1
Coma	1
Seizures[b]	1
Cyanosis	1
Respiratory insufficiency[c]	2
Hypertension (147/91–240/120 mmHg)[d]	5
Tachycardia (102–108/min)	4
Abdominal pain	2
Nausea	2
Vomiting	5
Diarrhea	2
Severity of poisoning[e,f]	Grade 2 (5 patients)
	Grade 3 (2 patients)[a]
	Grade 4 (2 patients)[c]

[a] In three patients the muscle weakness and dyspnea required observation in ICU; two were mechanically ventilated.
[b] Brief, resolved spontaneously, normal brain CT, no EEG was performed.
[c] These patients required mechanical ventilation.
[d] In two more hypertensive patients this problem was previously diagnosed.
[e] According to the classification of Fukuda and Tani (Halstead, 1988; Isbister and Kiernan, 2005; Isbister, 2004): grade 1, perioral numbness and paraesthesias, with or without gastrointestinal symptoms; grade 2, lingual numbness, numbness of face and other areas, early motor paralysis and incoordination, slurred speech, normal reflexes; grade 3, generalized flaccid paralysis, respiratory failure, aphonia, fixed/dilated pupils, conscious patient; grade 4, severe respiratory failure and hypoxia, hypotension, bradycardia, cardiac dysrhythmias, unconsciousness may occur.
[f] Two patients who had a very small taste of the fish liver and one who ate its musculature remained asymptomatic

Bentur et al., 2008 – Israel. Thirteen patients, presented between 2005- 2008

Elshama et al., 2011 – Egypt
Nine patients admitted to General Suez Hospital, Suez City, Red Sea.

Table (3): Relationship between clinical grading system of puffer fish poisoning, onset of symptoms, eaten part of fish and prognosis of all intoxicated patients

Patient	Onset of symptoms (Minutes)	Grade	Eaten part of fish	Prognosis	Cause of death
1	>30	4	Gonads+ flesh	Death	R.F
2	>30	1	Flesh	Recovery	-
3	>30	2	Flesh	Recovery	-
4	<30	1	Flesh	Recovery	-
5	30-60	4	Head + Flesh	Death	R.F
6	30-60	2	Soup + Flesh	Recovery	-
7	30-60	2	Flesh	Recovery	-
8	<30	4	Head + Flesh	Death	RF
9	<30	1	Flesh	Recovery	-

R.F: Respiratory failure

Clinical picture was divided into grading according to clinical grading system Wan et al., (2007). Grade 1 is characterized by perioral numbness and paraesthesia while grade 11 shows numbness of tongue, face and other areas, incoordination and slurred speech. Grade 111 is manifested by flaccid paralysis, dyspnoea and aphonia . Grade 1V is respiratory failure and coma.

[Wan C.K; Tsui S.H and Tong H.K .,2007 : A case series of puffer fish poisoning. Hong Kong j.emerg.med.;14:215-220.]

ANNEX VIII Projection of climatic suitability for *Lagocephalus sceleratus* establishment

Aim

To project the climatic suitability for potential establishment of *Lagocephalus sceleratus* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF), Atlas of Living Australia, Ocean Biogeographic Information System (OBIS), VertNet, iDigBio, and from a database of literature reports of occurrence in the Mediterranean (Argyro Zenetos, *pers. comm.*). The occurrence records were scrutinised to remove those older than 1950, appearing to be dubious or having overly imprecise georeferencing. Five records at outlying depths greater than 200 m were removed, since the species is known to be restricted to shallow water. The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling (Figure 1a). This resulted in 707 grid cells containing records of *L. sceleratus* for the modelling (Figure 1a), which is an adequate number for distribution modelling.

Climatic predictor variables were derived from the Bio-Oracle2 database of marine environmental layers (Tyberghein et al., 2012; Assis et al., 2018) 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. Consideration of the likely limiting factors on establishment in European waters led to selection of the following climate variables being used in the modelling:

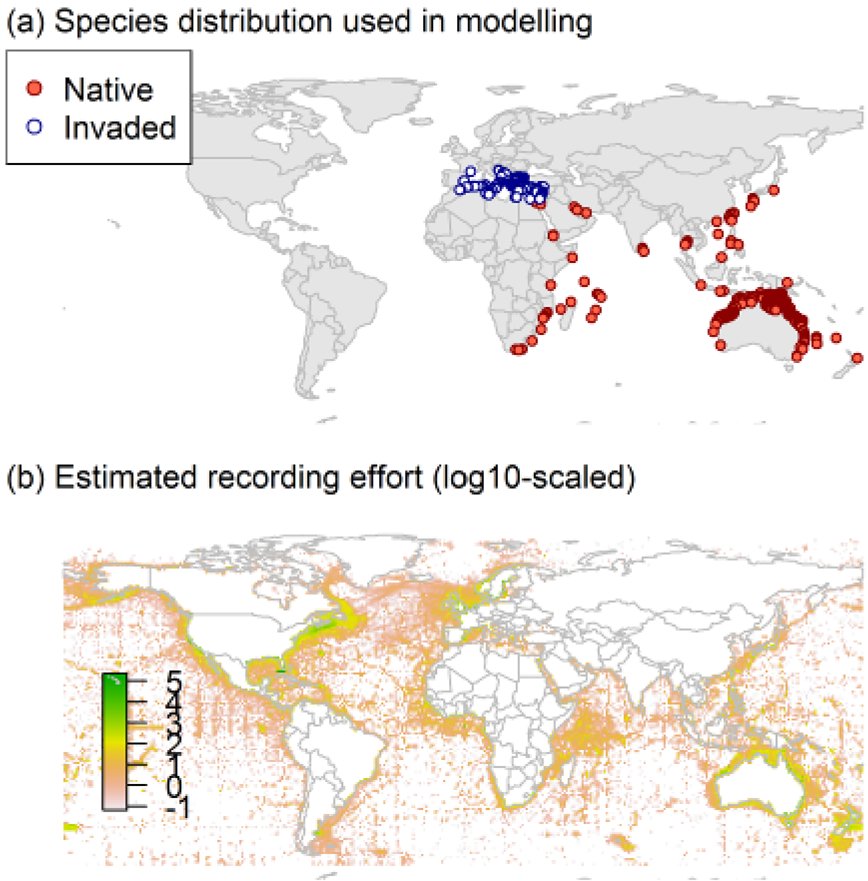
- <u>Minimum depth</u> (m, log+1 transformed) derived from the maximum bathymetry layer of Bio-Oracle (i.e. minimum depth) and aggregated to the shallowest depth. *L. sceleratus* requires shallow water in order to spawn, preferably on *Posidonia oceanica* meadows in the Mediterranean but also on sandy or algae-covered rocky habitats.
- <u>Maximum temperature at maximum depth</u> (°C) since spawning may be limited by low temperature.
- <u>Minimum sea surface temperature</u> (°C) which may represent a constraint on adult and juvenile survival. Juveniles in particular persist in coastal areas throughout the year.

The only non-climatic predictor was <u>mean sea surface salinity</u> (PSS) as low salinity environments are not considered suitable. This layer was taken from the MARSPEC database (Sbrocco & Barber, 2013), as this was considered more reliable for the Mediterranean then Bio-Oracle.

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2050s under the Representative Concentration Pathway (RCP) 4.5 and 8.5 were also obtained from Bio-Oracle. RCP 4.5 is a moderate climate change scenario in which CO_2 concentrations increase to approximately 575 ppm by the 2050s and then stabilise, resulting in a modelled global temperature rise of 1.8 °C by 2100. For the 2050s scenario, water temperatures at the occurrences rise by an average of 0.8 °C. RCP8.5 is the most extreme of the RCP scenarios, and may therefore represent the worst case scenario for reasonably anticipated climate change. In RCP8.5 atmospheric CO_2 concentrations increase to approximately 850 ppm by the 2050s, resulting in a modelled global mean temperature rise of 3.7 °C by 2100. For the 2050s scenario, water temperatures at the occurrences rise by an average of 1.2 °C.

Future salinity scenarios were not available from MARSPEC, so these were approximated by calculating the expected change in salinity from Bio-Oracle and applying this change to the MARSPEC baseline.
Finally, the recording density of Actinopterygii on GBIF was obtained as a proxy for spatial recording effort bias (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Lagocephalus sceleratus* and used in the modelling, showing the native range and (b) a proxy for recording effort – the number of Actinopterygii records held by the Global Biodiversity Information Facility, displayed on a $\log_{10}$ scale.



Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7 (Thuiller et al., 2009, 2016). 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. Therefore background samples (pseudo-absences) were sampled from two distinct regions:

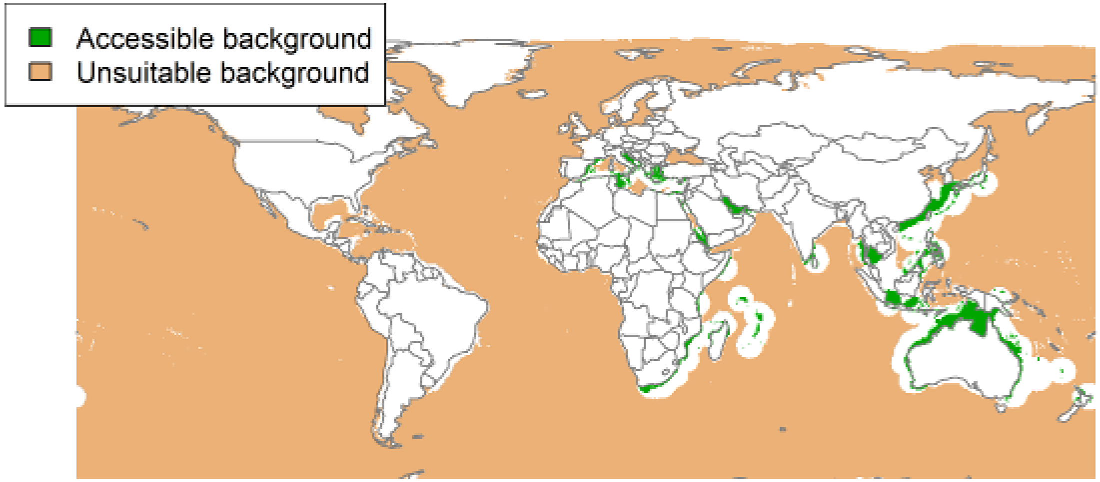
- An accessible background includes places close to *L. sceleratus* populations, in which the species is likely to have had sufficient time to disperse and sample the range of environments. *Lagocephalus sceleratus* is considered to be highly mobile and capable of swimming up to 250 km. Therefore we defined the accessible background as a 250 km buffer around non-native records, and a 500 km buffer around the native records. Sampling was more restrictive from the invaded range to account for stronger dispersal constraint over a shorter residence time.
- An unsuitable background includes places with an expectation of environmental unsuitability, e.g. places too cold or hot. Absence from these regions should be irrespective of dispersal constraints, allowing inclusion of this background in the modelling. No specific ecophysiological information was available to define the unsuitable region, but based on expert opinion that depth, cold and low salinity are likely to be limits on *L. sceleratus* occurrence in Europe unsuitability was defined as:
    - Minimum depth > 180 m, OR
    - Mean sea surface temperature < 16 °C, OR
    - Maximum temperature at maximum depth < 13 °C, OR
    - Mean salinity < 29 PSS.

This unsuitable background contained just 1% of the occurrences.
Ten replicate random background samples were obtained:

- From the accessible background 707 samples were taken, which is the same number as the occurrences. These were sampled with similar recording bias using the target group approach (Phillips, 2009) in which sampling of background grid cells was weighted in proportion to the Actinopterygii recording density (Figure 1b). Taking same number of background samples as occurrences ensured the background sample had the same level of bias as the data.
- From the unsuitable background 3000 simple random samples were taken. Sampling was not target group weighed as we are confident that these are reliable absences.

Model testing on other datasets has shown that this method is not overly sensitive to the choice of buffer radius for the accessible background or the number of unsuitable background samples.

Figure 2. The background regions from which 'pseudo-absences' were sampled for modelling. The accessible background is assumed to represent the range of environments the species has had chance to sample. The unsuitable background is assumed to be environmentally unsuitable for the species.



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 (except where specified below) and rescaled using logistic regression:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per effect.
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent (Phillips et al., 2008)

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 calculating the Area Under the Receiver-Operator Curve (AUC) for model predictions on the evaluation data, which were reserved from model fitting. AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected pseudo-absence.

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.

Global model projections were made for the current climate and for the two climate change scenarios, avoiding model extrapolation beyond the ranges of the input variables. The optimal threshold for partitioning the ensemble predictions into suitable and unsuitable regions was determined using the 'minimum ROC distance' method. This finds the threshold where the Receiver-Operator Curve (ROC) is closest to its top left corner, i.e. the point where the false positive rate (one minus specificity) is zero and true positive rate (sensitivity) is one.

Limiting factor maps were produced following Elith et al. (2010). 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. Partial response plots were also produced by predicting suitability across the range of each predictor, with other variables held at near-optimal values.

Results

The ensemble model suggested that at the global scale and resolution of the model suitability for *L. sceleratus* was most strongly determined by temperature at maximum depth, depth and sea surface temperature (Table 1, Figure 3).

Global projection of the ensemble model in current climatic conditions indicates that the native and invaded records generally fell within regions predicted to have high suitability (Figure 4). In Europe, nearly all of the Mediterranean coastal region was predicted to be suitable for invasion (Figure 5), with higher suitability in the eastern Mediterranean than the west because of cooler sea surface temperatures (Figure 6). The central Mediterranean was predicted to be too deep for establishment (Figure 6). Outside of the Mediterranean, limited invasion of the Atlantic coast of southern Spain and Portugal may be possible, but low sea surface temperature is predicted to prevent northwards spread into the Atlantic (Figure 6). Invasion of the Black Sea was predicted to be prevented by low temperature (Figure 6) although the Black Sea also has very low salinity. Since *L. sceleratus* is still expanding its range towards the Atlantic and Black Sea and northwards into the Adriatic and meeting novel environmental conditions at those invasion fronts, the precise location of the potential range margin is difficult to predict.

By the 2050s, under the moderate RCP4.5 and extreme RCP8.5 climate change scenarios, suitability in the Mediterranean increases, but the model predicts little potential expansion of the suitable region into the Atlantic of the Black Sea (Figures 7-8).

Table 1. Summary of the cross-validation predictive performance (AUC) and variable importances of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to ten different background samples of the data.

Algorithm	AUC	In the ensemble	Variable importance			
			Minimum depth	Mean sea surface temperature	Maximum temperature at maximum depth	Mean surface salinity
GBM	0.9653	yes	20%	28%	49%	4%
Maxent	0.9625	yes	29%	18%	36%	17%
ANN	0.9615	yes	28%	29%	41%	2%
GAM	0.9608	yes	41%	26%	29%	4%
MARS	0.9598	yes	27%	21%	47%	5%

GLM	0.9582	no	37%	24%	32%	6%
RF	0.9543	no	12%	18%	59%	11%
Ensemble	**0.9659**		**29%**	**24%**	**40%**	**6%**

Figure 3. Partial response plots from the fitted models, ordered from most to least important. 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.

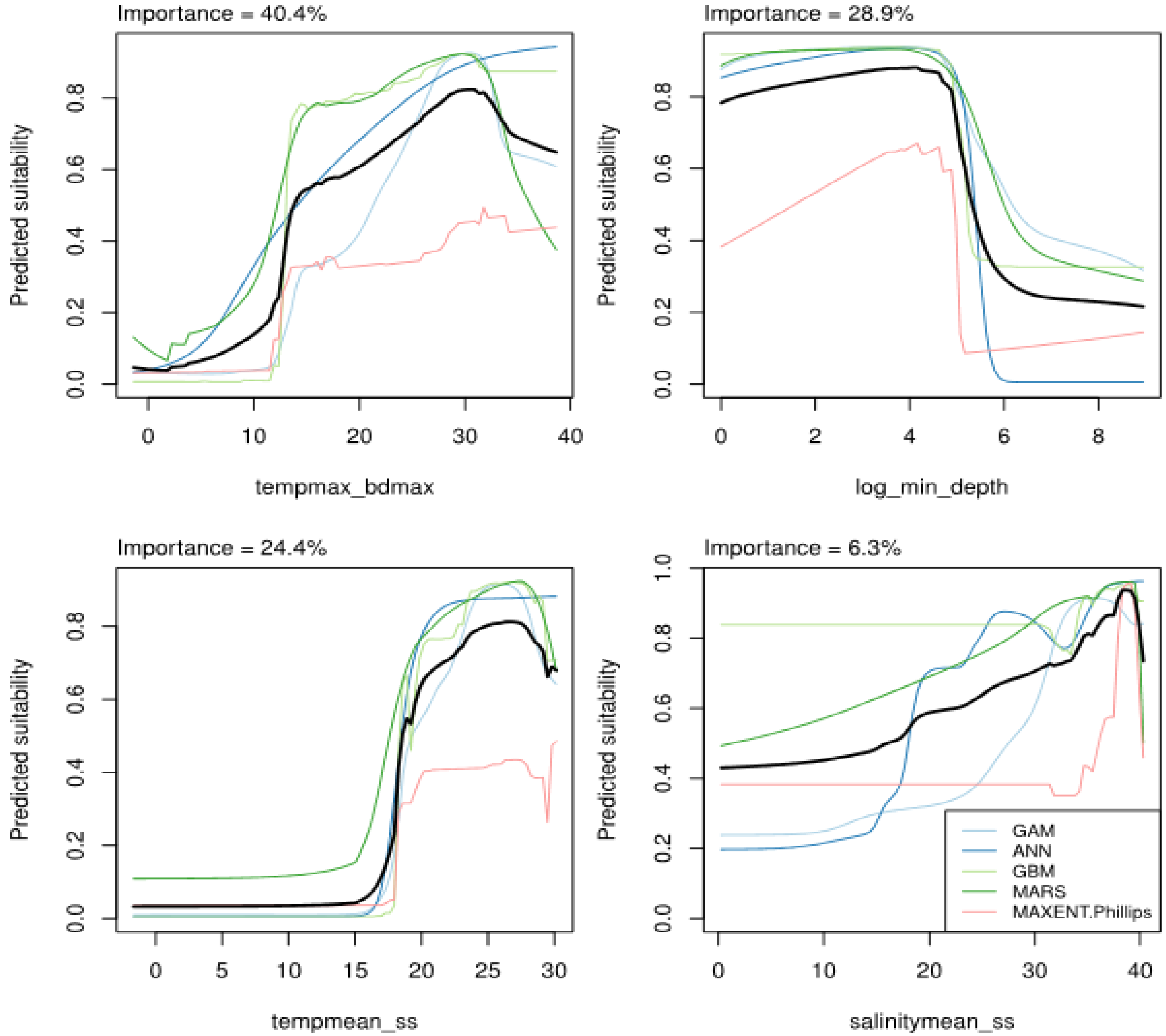


Figure 4. (a) Projected global suitability for *Lagocephalus sceleratus* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Red shading indicates suitability. White areas have climatic conditions outside the range of the training data so were excluded from the projection. (b) Uncertainty in the suitability projections, expressed as the standard deviation of projections from different algorithms in the ensemble model.

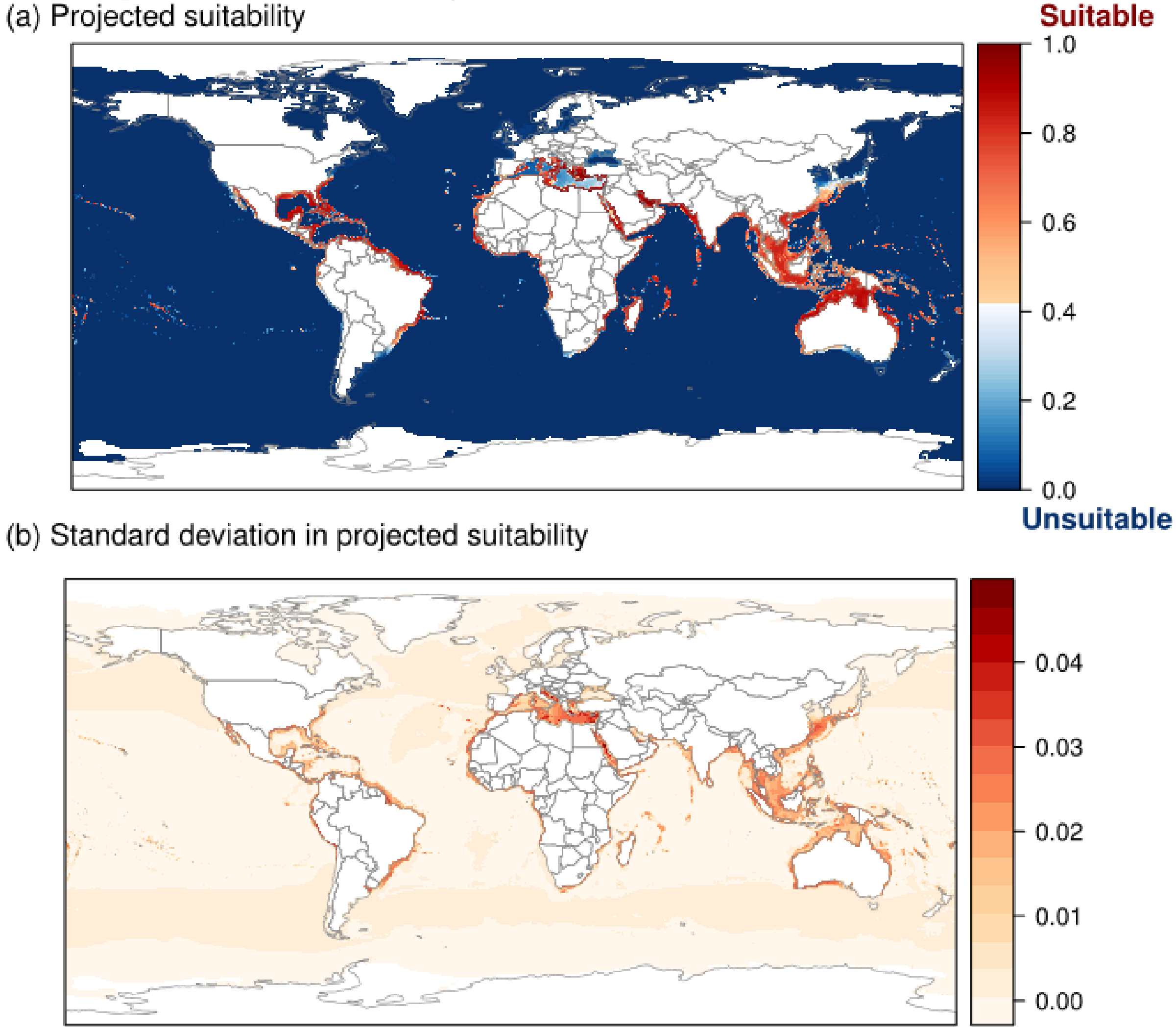


Figure 5. Projected current suitability for *Lagocephalus sceleratus* establishment in Europe and the Mediterranean region. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

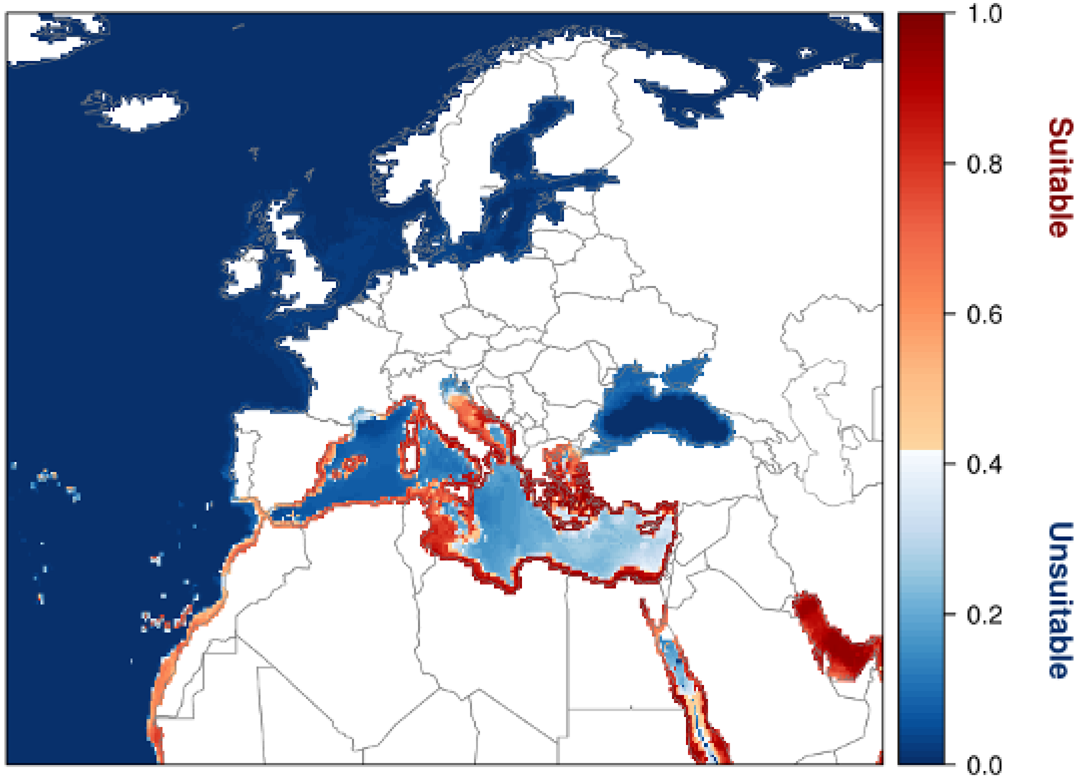


Figure 6. Limiting factor map for *Lagocephalus sceleratus* establishment in Europe and the Mediterranean region in the current climate. Shading shows the predictor variable most strongly limiting projected suitability.

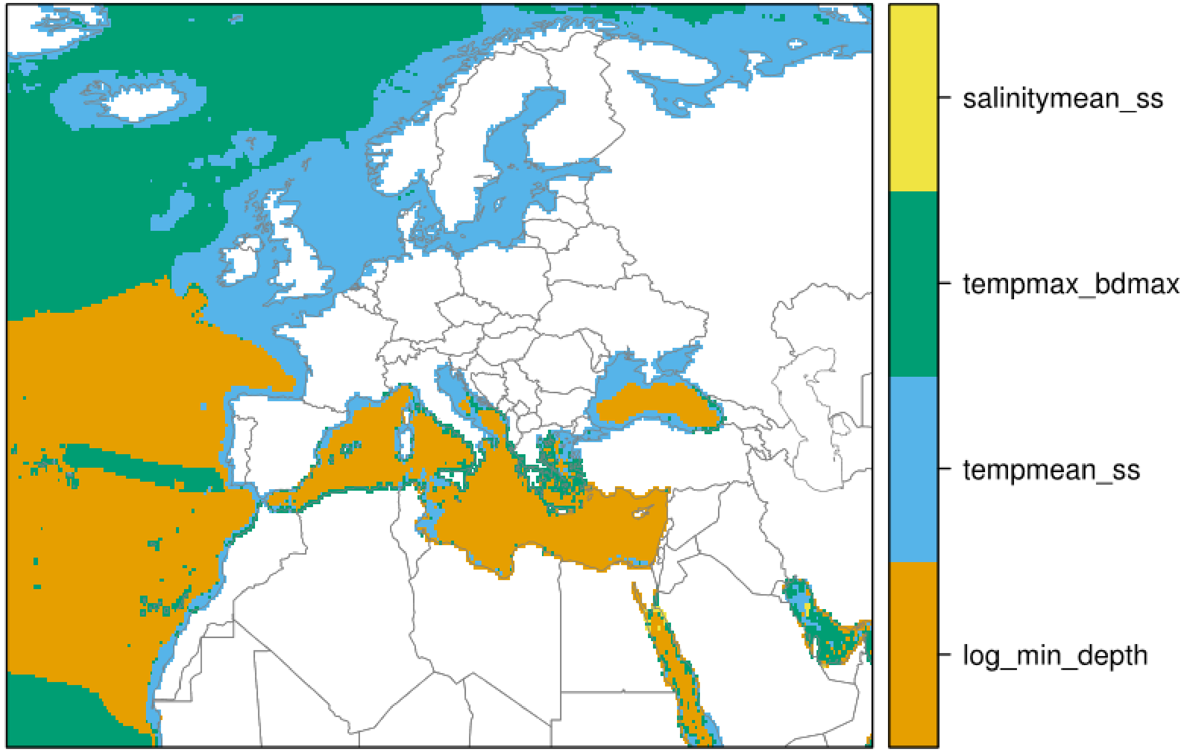


Figure 7. Projected suitability for *Lagocephalus sceleratus* establishment in Europe and the Mediterranean region in the 2050s under climate change scenario RCP4.5, equivalent to Figure 5.

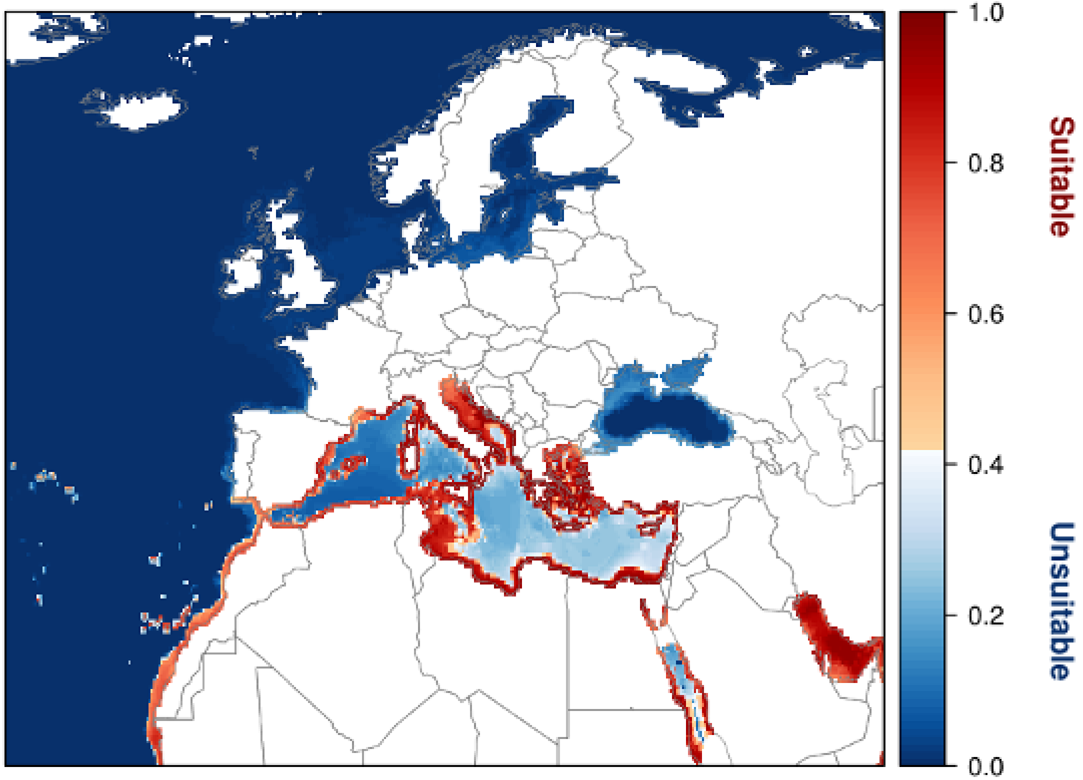
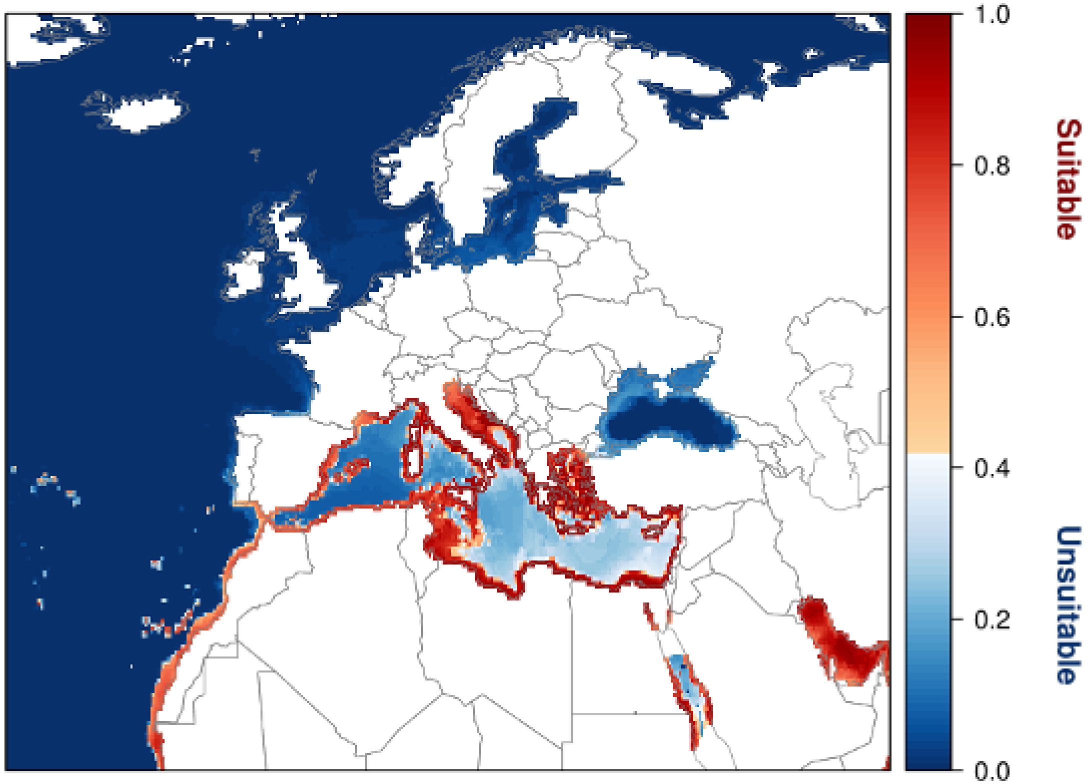


Figure 8. Projected suitability for *Lagocephalus sceleratus* establishment in Europe and the Mediterranean region in the 2050s under climate change scenario RCP8.5, equivalent to Figure 5.



Caveats to the modelling

Modelling the potential distributions of range-expanding species is always difficult and uncertain.

The modelling here is subject to uncertainty for the following reasons:

- There was no ecophysiological information available to contribute to definition of the unsuitable background region.
- *Lagocephalus* species are known to be adaptable and may be able to expand their niche into cooler conditions than are currently observed, extending the region at risk of invasion. Indeed, the expansion into the western Mediterranean represents exposure to cooler waters than are

experienced in the native range, suggesting that the model might under-predict the potential range in the Atlantic, Adriatic and Black Sea or Sea of Marmara.

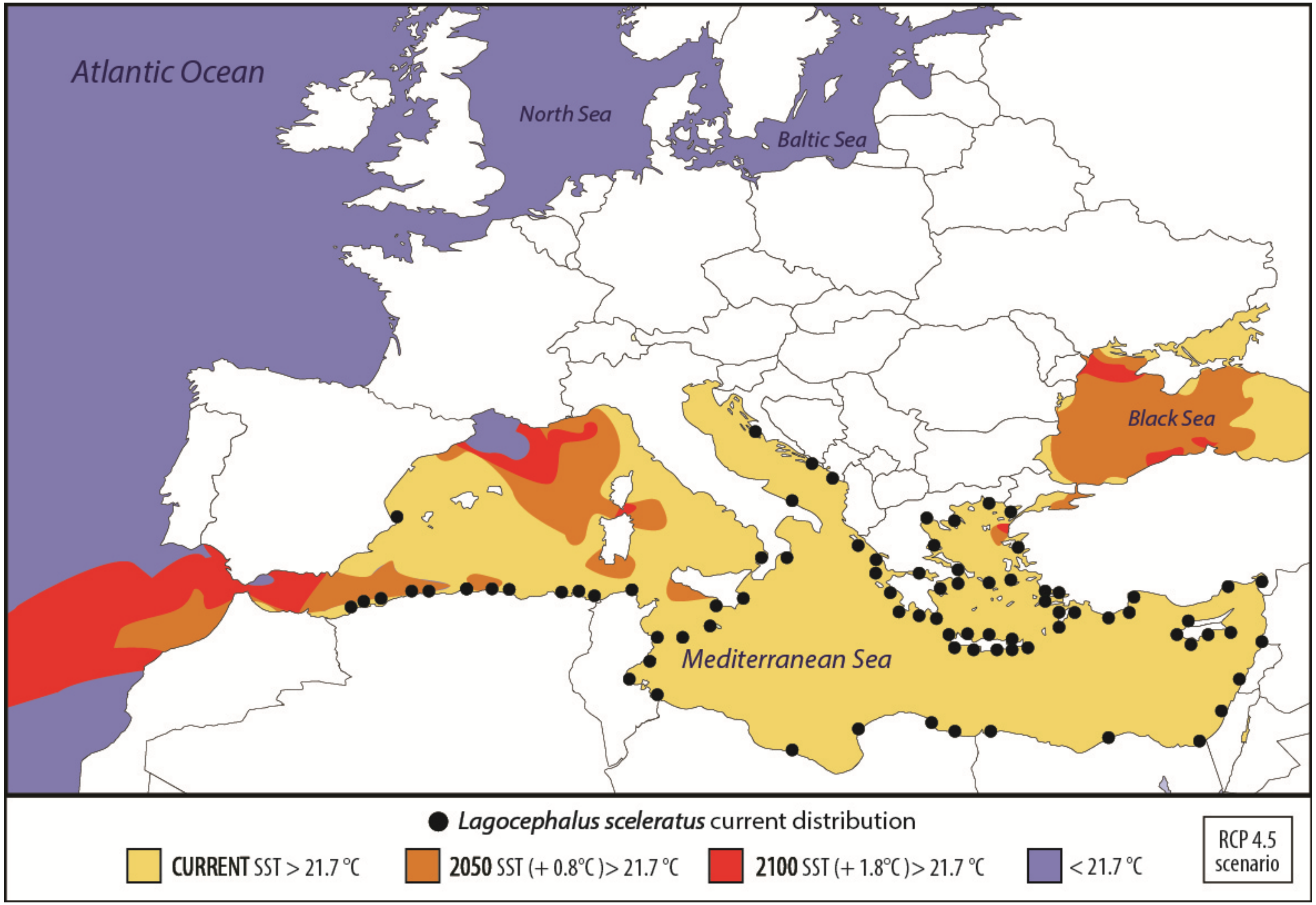
- The predictor variables derived from Bio-Oracle and MARSPEC are themselves subject to uncertainty which will propagate into the modelled species-environment relationships and distribution projections.

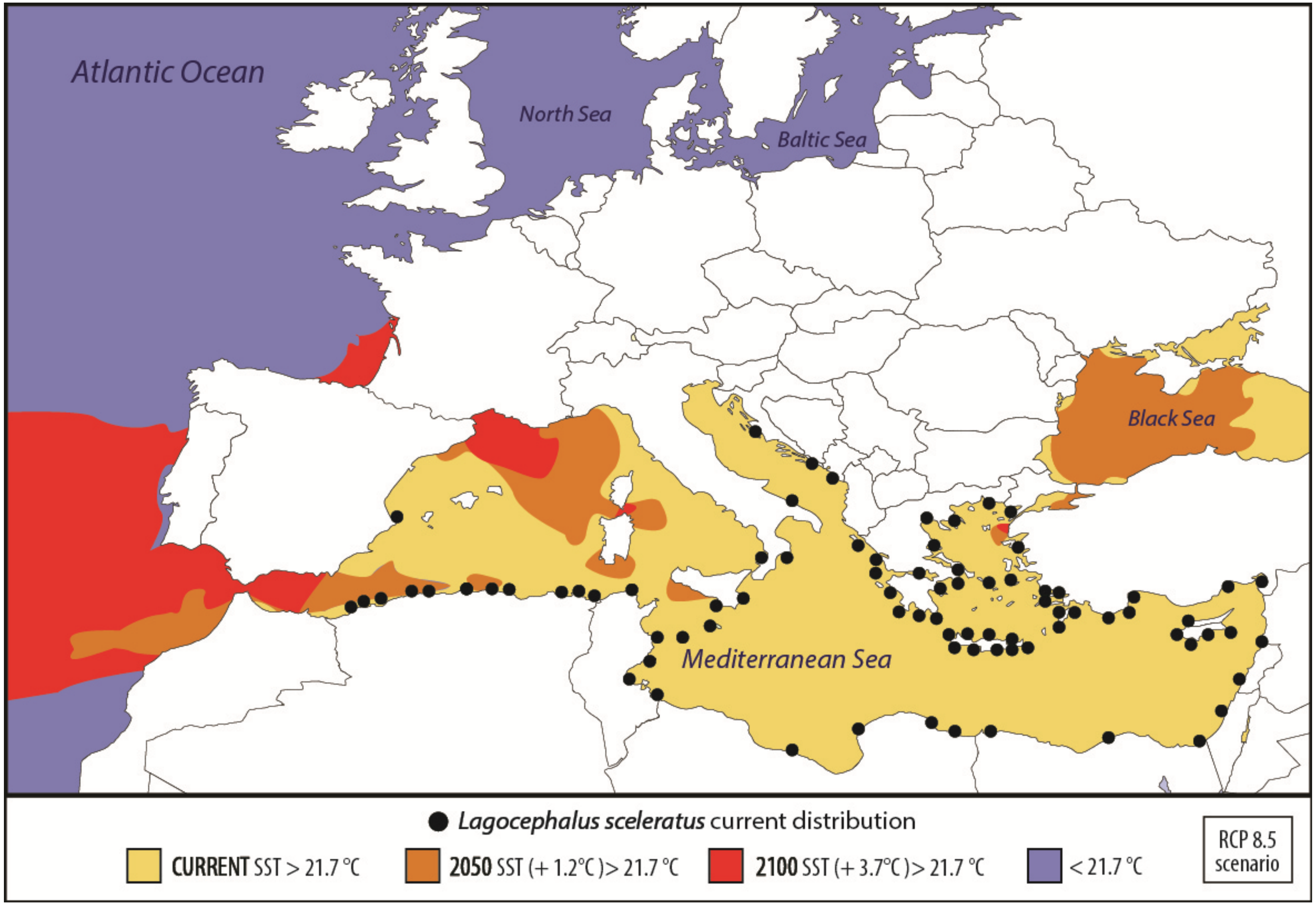
The model did not include other variables potentially affecting occurrence of the species, including spawning habitat availability or biotic interactions.
To remove spatial recording biases, the selection of the background sample was weighted by the density of Actinopterygii records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, this may not have been the perfect model for species recording effort, especially because additional data sources to GBIF were used.

Additional information on spawning requirements (not included in the model)
Literature on the con-generic *Lagocephalus lunaris spadiceus* (Fujita, 1966) and the distribution of spawning aggregations of *L. sceleratus* in southern Cyprus (Michailidis, 2010; Rousou et al., 2014) indicate that there might a thermal limit for spawning at around 21-22 °C SST in June (peak spawning month). A threshold of 21.7 °C SST for the month of June was tentatively applied as a limiting factor for spawning (value taken from Fujita, 1966). Data for monthly SST means between 2012-2016 were retrieved from http://marine.copernicus.eu/services-portfolio/access-to-products/?option=com_csw&view=details&product_id=GLOBAL_ANALYSIS_FORECAST_PHY_001_024
This data was not included in the modeling because the particular dataset does not include future climate projections, it was used however as supporting information for the prediction of the potential distribution of the species. The resulting map (Note that there is no depth limit imposed) shows that all but one of the current observations in the invaded range fall within this thermal limit and that certain areas in the western Mediterranean may not favour the reproduction of the species. This particular value however was derived from laboratory experiments, where eggs of a con-generic hatched at temperatures between 21.7-24.5 °C and may not accurately represent the physiological requirements of *L. sceleratus* in the RA area, particularly since the species is already showing signs of adaptation to cooler waters compared with the native distribution. It may be perceived though as a relative measure of recruitment strength, as it is considered unlikely that a species will thrive in large numbers at the boundaries of areas of habitat suitability even though it may be present (Townhill et al., 2017). Similar maps were produced for the RCP4.5 and RCP8.5 scenarios with the following approximations

RCP4.5 2050	current June SST + 0.8 °C
RCP4.5.2100	current June SST + 1.8 °C
RCP8.5 2050	current June SST + 1.2 °C
RCP8.5 2100	current June SST + 3.7 °C

Care should be taken when interpreting these maps that they are binary (i.e. contain only two classes) and they represent only one parameter; they do not reflect the other possible limiting factors in the RA area (e.g. low salinity and winter temperature in the Black Sea, low winter temperature in the North Adriatic, etc.) or the adaptability of *L. sceleratus* to colder temperatures.


Atlantic Ocean
North Sea
Baltic Sea
Black Sea
Mediterranean Sea
Lagocephalus sceleratus current distribution
CURRENT SST > 21.7 °C
2050 SST (+ 0.8°C)> 21.7 °C
2100 SST (+ 1.8°C)> 21.7 °C
< 21.7 °C
RCP 4.5 scenario


Atlantic Ocean
North Sea
Baltic Sea
Black Sea
Mediterranean Sea
Lagocephalus sceleratus current distribution
CURRENT SST > 21.7 °C
2050 SST (+ 1.2°C)> 21.7 °C
2100 SST (+ 3.7°C)> 21.7 °C
< 21.7 °C
RCP 8.5 scenario

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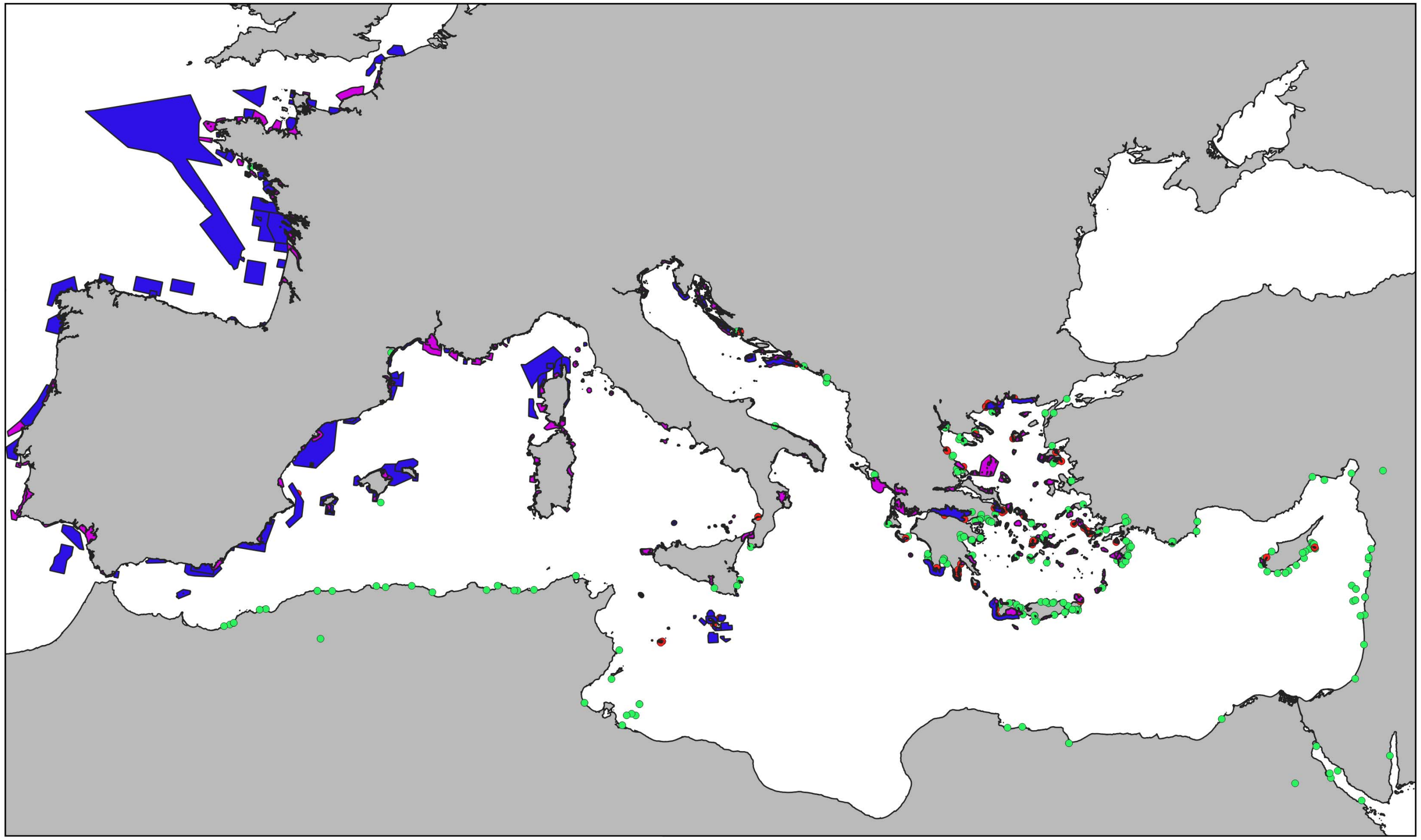
ANNEX IX. Question to European Union Aquarium Curators (EUAC) members

Maria Corsini-Foka, HSR-HCMR, 14/6/18					
Question to European Union Aquarium Curators (EUAC) members and other Aquarium curators: Do you display Lagocephalus sceleratus?: YES-NO					
Email posted by M. Corsini-Foka on 7/6/18					
114 email addresses linked					
Out of office: 10					
Name of Curator/EUAC members	**Aquarium**	**Country**	**YES**	**NO**	**Remarks**
Maria Corsini-Foka	Aquarium Rhodes	Greece	YES, since 2007, sometimes		Corsini-Foka et al., 2014, CBM
Nuria Baylina and Nuno Vasco Rodrigues	Oceanário de Lisboa	Portugal		NO	
LARS SKOU OLSEN	Den Blå Planet Danmarks Akvarium-Kastrup	Denmark		NO	
Jean Philippe CATTEAU	MARINELAND PARCS-ANTIBES CEDEX	France	Yes, in 2014		
Stéphane AUFFRET	Ocearium Le Croisic	France		NO	
Stefane Farkasdi	? Kolmarden Tropicarium	Sweden		NO	
Marion Wille	Aquazoo Löbbecke Museum-Düsseldorf	Germany		NO	
Attila Varga	Sosto Zoo /Nyíregyházi Állatpark Nonprofit Kft.-Nyiregyhaza-	Hungary		NO	

	Sostofürdö				
Fátima Santos Gil	Aquario vasco de Gama-Lisboa	Portugal		NO	
Ester Alonso	Loro Parque-Tenerife	Spain		NO	
James Wright	National marine Aquarium-Plymouth	UK		NO	
Thomas Jermann	ZOOLOGISCHER GARTEN BASEL	Switzerland		NO	
Daniel Abed-Navandi	Haus des Meeres-Vienna	Austria		NO	L. lagocephalus, in 2011, provided by "Flyingshark"
Primo Micarelli	Aquarium Mondo Marino-Massa Marittima	Italy		NO	
Pablo Montoto Gasser	ZooAquarium de Madrid	Spain		NO	
Brian Zimmerman	Zoological Society of London	UK		NO	
Aspasia Sterioti	CRETAQUARIUM	Greece	Yes, since 2009		
Mark de Boer	Rotterdam Zoo	Netherlands		NO	
Max Janse	Burger Zoo	Netherlands		NO	
Isabel Koch	Wilhelma der zoologisch-botanische Garten-Stuttgart	Germany		NO	
Philippe Jouk	Antwerp Zoo Aquarium	Belgium		NO	
Jakub Kordas	Kierownik ds. akwariów/ Afrykarium ZOO Wrocław	Polland		NO	
Pierre MORINIERE	aquarium LA ROCHELLE	France		NO	
Amalia Martínez de Murguía	Aquarium Donostia-San Sebastián	Spain		NO	
Lebedev Victor	? Oceanarium Russian Federal Research Institute of Fishery and Oceanography (VNIRO) Moscow?	Russia		NO	
Alberto Castellanos	Acuario Poema del Mar-Las Palmas de Gran Canaria	Spain		NO	*L. lagocephalus*, few months ago
Olivier BRIARD	Aquarium Biarritz	France		NO	
Nicolas Hirel	Planet Ocean Montpellier (former Aquarium Mare Nostrum	France		NO	
Olivier BRUNEL	Aquarium Institut océanographique, Fondation Albert Ier, Prince de	Monaco		NO	

	Monaco				
Christian Michel and Marie Bournonville	Aquarium of Liege University	Belgium		NO	
RUNE KRISTIANSEN	Basta Aquarium Kattegatcentret	Denmark		NO	
Julia Duhem	Pairi Daiza-Brugelette	Belgium		NO	
Anke Oertel	Haus der Natur Museum für Natur und Technik-Salzburg	Austria		NO	
Patrici Bultó	L' Aquàrium de Barcelona	Spain		NO	
Total	**34**		**3**	**31**	
Other Info/Not EUAC					
	Alexandria Aquarium Egypt	Egypt	Yes		Corsini-Foka et al., 2014, CBM
	Ocean Aquarium Cyprus	Cyprus	Yes		Corsini-Foka et al., 2014, CBM
	Yes, today	Yes, in past	Never		
N. of Public Aquaria	**4**	**1**	**23**		

ANNEX X. *L. sceleratus* within Natura2000 sites



Lagocephalus over Natura2000 sites

Natura Sites with marine
100% marine
<100% marine

Lagocephalus sceleratus 29.6.18
L. sceleratus over Natura sites

ANNEX XI. DATA FOR SMALL SCALE COASTAL FISHERIES OF CYPRUS FOR THE YEARS 2001 to 2013.

Source: Cyprus Department of Fisheries and Marine Research (DFMR) Annual Reports 2001-2013.

LANDINGS (t)	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
octopuses	114.94	65.18	47.94	53.49	80.05	91.61	137.89	102.62	25.75	24.82	36.79	33.63	45.16
squid & cuttlefish	77.40	70.68	54.81	50.14	72.13	77.54	72.90	54.65	35.93	37.40	31.59	29.75	28.65

EFFORT	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
boats	500	500	500	500	500	457	490	500	496	500	500	465	473
LOA	4015	4136	4034	4042	4074	3756	3974	4084	4073	4094	4085	3827	3893
KW	16085	19479	17714	17620	19384	17923	18741	19600	22419	23018	23416	21841	22074
CPUE per boat													
	2001	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
octopuses	0.23	0.13	0.10	0.11	0.16	0.20	0.28	0.21	0.05	0.05	0.07	0.07	0.10
squid & cuttlefish	0.15	0.14	0.11	0.10	0.14	0.17	0.15	0.11	0.07	0.07	0.06	0.06	0.06

Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	***Lagocephalus sceleratus*** **(Gmelin, 1789)**
Species (common name)	**Silver-cheeked toadfish, silver-stripe blaasop**
Author(s)	**Marika Galanidi, Argyro Zenetos**
Date Completed	**23.9.2018**
Reviewers	**Jack Sewell, Elena Tricarico, Peter Robertson**

Summary
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 Lessepsian immigrant, already established in parts of the Mediterranean Sea and spreading unaided towards its western and northern basins, very little, if anything can be done to prevent further introductions through natural dispersal. Management at the Suez Canal level, although the only possible solution for lessepsian migration, does not seem to be a realistic expectation and is outside the jurisdiction of EU Member States. Due to its high toxicity and already manifested socio-economic and environmental impacts in the East Mediterranean, the species is high on the radar of competent authorities and scientific/stakeholder/citizen scientist networks both for purposes of early detection and for awareness raising of the risk it poses to human health. Invasive species platforms and initiatives such as MedMIS, SeaWatchers are already contributing to the early detection of *L. sceleratus*, while monitoring can be achieved with survey programmes (e.g. the MEDITS surveys, FAO/GFCM activities) and commercial fishing activities. Eradication of this species is acknowledged to be impossible due to the already widespread and abundant populations, the high fecundity, mobility, long pelagic duration of the early life stages of the species and its spawning on sensitive habitats. Direct removal with intensive targeted fishery has been implemented in Cyprus without any evidence of suppressing the populations of *L. sceleratus*, it does however provide some financial compensation to small-scale fishermen who suffer significantly from gear, labour and catch losses due to this species. Appropriate modifications to different fishing gears are already being applied by fishermen and experimented on by fisheries scientists such that damages can be minimised and the fishing gears can become more effective in capturing *L. sceleratus*. As an alternative to fisheries removals, mass trapping with pheromones may be considered a promising approach, one that is more species-specific and less damaging to the environment compared to less selective, more invasive removal methods. This would be however a long, complex and costly process that requires extensive laboratory and field experimentation. Two other possible

measures for the management of the species include harvesting for commercial purposes. One is the exploitation for pharmaceutical research and applications of the TTX toxin contained in *L. sceleratus* tissues. The cost-effectiveness of harvested TTX, compared with its synthetic production needs to be properly evaluated for the Mediterranean populations. The second measure includes the depuration of *L. sceleratus* from TTX for human consumption, a process that has been implemented successfully in congeneric, edible, less toxic pufferfish species. Besides the high requirements in space, time and investment of such an undertaking, public concern about the toxicity of the species before depuration will most likely make sale/marketing problematic. Moreover any control / management measure should be carefully implemented as any new markets created by the action would generate pressure for fisheries to be sustained and pressure against overfishing and 'overexploitation' of the species.

Detailed assessment			
	Description of measures	**Assessment of implementation cost and cost-effectiveness (per measure)**	**Level of confidence**
Methods to achieve prevention	**P1**. Installation of high-salinity locks in the Suez Canal (Goren & Galil, 2005) / Reinstating the former salinity barrier of the Bitter Lakes (Galil et al., 2017)	This is a major technical and financial undertaking, requiring international co-operation. Edelist et al. (2013) consider this a highly impractical suggestion. However, despite the recent enlargement (2015) and no salinity barrier, the number of new introductions in the Mediterranean via the Suez appears to be declining after 2015 (Zenetos, 2017)	**Medium** The Suez Canal barrier, was removed as of the 1970s, when the Nile River flow to the Mediterranean was arrested by the Aswan dam (Rilov and Galil, 2009) and this led to an increased influx of species in the Mediterranean (Safriel, 2013). The Panama Canal constitutes a physical and mostly physiological barrier (a freshwater lake) that proved to be much more hostile to a transport stage compared to the

			Suez Canal barrier (Por 1978).
	-- Policy co-ordination at the regional level, including non EU states (Barcelona Convention).	--- To date, proposed management measures for species introductions in the Mediterranean in the framework of the Barcelona convention have excluded introductions via the Suez Canal (Galil et al., 2016). Moreover, in the framework of the Marine Strategy Framework Directive, Descriptor 2 (Non-indigenous species), species entering through the Suez Canal are excluded from indicator 2.1.1 (i.e.trends in abundance, temporal occurrence and spatial distribution in the wild of non-indigenous species, particularly invasive non-indigenous species, notably in risk areas, in relation to the main vectors and pathways of spreading of such species). COMMISSION DECISION (EU) 2017/848. See also Palialexis et al. (2015).	-----------------------------
	P2. To prevent ESCAPE from confinement from open or semi-open circulation aquaria: stricter control/enforcement of cleaning operations (filters, disinfection), especially at the outlet to the sea	Improved biosecurity measures in public and research aquaria would address a potential pathway for a range of species. Article 3 of the EU Zoos Directive recognizes that for aquatic species, it is paramount to prevent incidental escapes from the water and offers guidance for best practices. A first line of actions is to secure enclosures against animal escape. In large public aquaria, circulation systems are closed.	Medium

Methods to achieve eradication (E1-E3)	Eradication of species in the marine environment is difficult. Theoretically, eradication may be possible for localised, newly established populations at low densities with limited dispersal capabilities (Delaney & Leung, 2010; Ojaveer et al, 2015). This would require an early warning system, monitoring efforts and a removal program.	Physical removal of invasive species is generally endorsed by informed stakeholders, as long as it can easily be stopped and has no long-term consequences for the marine environment (Thresher & Kuris 2004) In the marine environment, eradication of naturally dispersing species is generally considered unrealistic and has only been achieved in a handful of cases, when the introduced species had sessile adult stages, the populations were small and restricted, human and financial resources were available, and early action was taken (Williams & Grosholz, 2008). This is clearly not the case for *L. sceleratus*, whose eradication is acknowledged to be impossible due to the already widespread and abundant populations, the high fecundity, mobility, long pelagic duration of the early life stages of the species and its spawning on sensitive habitats. Additionally, local eradication would require ongoing, long-term, regular interventions due to the ongoing risk of re-introduction and spread from surrounding populations or through the Suez Canal. Nevertheless, population control that leads to minimising the severity of impacts and the risk of transfer to yet uncolonised areas is considered feasible (Ojaveer et al., 2015).	High
	E1. Early warning systems / awareness raising The extreme toxicity of *L. sceleratus* prompted a fast response from competent Authorities at the EU and the national levels with awareness	 This has contributed to the early detection of the species as it expands its range, as attested by the continuous reporting of casual records in the Adriatic (e.g. Azzurro et al., 2016a), Malta (Deidun et al, 2015; Andaloro et al.,	 High

	raising campaigns and landing/marketing bans (see Q2.7; Ben Souissi et al., 2014 and references therein; Azzurro et al., 2016a). At the moment early detection systems operate through official and unofficial networks of national experts with local stakeholders and through official authorities in many Mediterranean countries [Spot the alien fish (Malta, Italy), iSea (Greece), RAC (Alien Corsican Network: France), relevant competent Authorities – see references for the relevant links].	2016), the Sea of Marmara and the western Mediterranean, (e.g. Tunisia – Ben Souissi et al., 2014). (see also Q 2.7). Another tool for effective knowledge exchange.is the network of networks (INVASIVESNET) which aims to facilitate greater understanding and improved management of invasive alien species (IAS) and biological invasions globally (Lucy et al., 2016). Re-activation of the MAMIAS (Marine Mediterranean Invasive Alien Species) platform to promote regional co-ordination and dissemination of data and important information.	
	E2. Monitoring Monitoring can be achieved through scientific (e.g. MEDITS International bottom trawl survey in the Mediterranean Sea) and fisheries dependent surveys and should focus on areas of anticipated expansion (i.e. Mediterranean Spain and France, North Adriatic, South Portugal). Monitoring throughout the invaded range is also recommended in order to follow the development of the species populations (critical for the manifestation of impacts) and allow	A cost-effective method, utilising existing survey programmes and commercial fishing activities. Additional monitoring activities may be necessary for year-round population studies. The spread of the species is also being monitored through citizen science/stakeholder engagement programmes (see E1). However, early warning and monitoring measures currently in place refer only to adult *L. sceleratus* and do not cover the early life stages. *L. sceleratus* is already included in the priority list of non-indigenous species for monitoring in relation to fisheries in the East Mediterranean in a pilot study by FAO/GFCM (UNEP/MAP, 2017). The proposal is that	High NGOs in the Mediterranean include it among target species to be reported.

	detection of reproductive areas, which is crucial information for the removal program.	the species is monitored through the Data Collection Reference Framework (DCRF) (CFP requirement) of EU Member States and the discards monitoring program of the GFCM (GFCM – UNEP/MAP, 2018). At the moment these programmes do not offer the possibility for the systematic collection of quantitative data, which is currently one of the monitoring priorities for *L. sceleratus*	
	E3. Removal program - Direct removal with intensive targeted fishery, especially during the spawning period, possibly combined with a bounty program. Different fishing methods can be employed (purse seines, trawls, longlines, trammel and gill nets, even angling) with appropriate modifications to minimise gear damage.	Hakan-Kaykaç et al. (2017) determined that fyke nets, handline, longline (baited and without bait) and purse seine (when schooling) can be used for the catching of *L. sceleratus*. Considering the sharp and strong jaw structure, hooks should have a long shank and be made of thick material. It is also important to use steel wire Ø 0.40-0.50 in the snood section of the handline and longline fishing instruments. It is thought that using surrounding nets such as purse seine can be appropriate, especially during the spawning season of the species. In addition, High Modulus Polyethylene material can be used in the surrounding nets bend section where the fish are collected. Trawls were also demonstrated to be an efficient gear for the catch of *L. sceleratus* (Öndes et al., 2017), this method however is not recommended as it goes against EU Regulation 1967/2006, which bans trawling at depths shallower than 50m throughout the year in the Mediterranean and additional fisheries restrictions implemented nationally in EU countries, mostly in the spring and summer months, to protect spawning stocks of commercial and other protected species. Thus, trawling would be destructive for native species and habitats,	Medium

		particularly sensitive habitats, such as reefs and seagrass beds.	
	---	---	-------------------------------
	Use a payment reward scheme (bounty system) to increase species removal	In order to control its populations, the government of Cyprus has been buying specimens from artisanal fishermen since 2012, for €3/kg, (a total cost of 600 K euro in five years: 2012-2016) through a management plan partly covered by European fisheries funds. The amount was set rather approximately but seems to satisfy fishermen. Delivered fish are being processed according to EU legislation (burned in a furnace). More than 150 tons have been fished and delivered so far, with no evident declining trend. (N. Michailidis, fisheries Dept, Cyprus, pers communication). However, the measure was implemented years after the species had already attained high populations in the region and had spread and established in neighbouring areas; its efficacy if implemented at an earlier time in the establishment of the species is uncertain. Depending on the selectivity of the removal method, targeted removal efforts can induce harvest-driven trait changes in invasive species (Závorka et al., 2018), e.g. by changing allometry, size at maturity, size distribution (Evangelista et al., 2015) with possible repercussions for the potential spread of the species. However, such impacts have not been studied in the invaded range to date. Mass-participation removal events (either in the form of “derbies” similar to the case of lionfish in the Caribbean, or linked to the bounty system) could also augment efforts for population control and damage limitation.	Medium
	---	---	------------------------------- Medium

	Development and deployment of pheromone traps to collect mature reproductive individuals.	A long, complex and involved process that requires extensive laboratory and field experimentation. Sex pheromones and fish aggregation pheromones have great potential for use in targeted trapping programs, especially for migratory nuisance species (Sorensen et al., 2016), such as *L. sceleratus* when spawning. However, identification and synthesis of individual semiochemicals (i.e. chemical compounds that convey information between and within species) has proven to be both difficult and expensive (Stewart and Sorensen 2015). When it comes to fish, only a small number of such compounds have been identified to date and the ones for sea lamprey (*Petromyzon marinus*) and carp species have been tested in the laboratory and the field (freshwater environment) with modest success (Sorensen et al., 2016). In the marine environment, among other challenges, the large scale will likely present additional difficulties. Advantages of mass trapping with pheromones include species-specificity and less damage to the environment compared to less selective, more invasive removal methods (El-Sayed et al., 2006). Additionally, this measure may prove more effective at controlling the development of large populations at earlier stages of the invasion.	
Methods to achieve management (M1 – M5)	**M1. Population control through targeted fishing activities.** If eradication is not possible at the core of the species' distribution, then further spread through natural dispersal will be very likely. However, it could still be theoretically possible to control/suppress the newly	Population control would most likely require a long-term commitment over consecutive years over localized areas (Barbour et al., 2011) and would involve a considerable cost. As with eradication campaigns, it may require changes in legislation on fishing restrictions and prove detrimental to other species and habitats. Furthermore, the example of Cyprus showed that intensive targeted fishery	Low

	established populations (Grosholz & Ruiz, 2002) with targeted fishing activities surrounding the core or new populations within a radius slightly larger than the yearly dispersal capability (Edwards & Leung, 2009).	with a bounty system has not been successful in suppressing populations when *L. sceleratus* has attained significant densities, even though it can provide some financial compensation to fishermen. Alternative methods need to be sought (see E3).	
	M2. Exploitation for pharmaceutical research and applications *L. sceleratus* is used for bioprospecting studies of its venom (see A.13 of the RA document) in various laboratories around the world and such studies/applications have been proposed for the Mediterranean invasive populations as well as a potential management measure to reduce populations of the species (e.g. Kosker et al., 2016; Nader et al., 2012).	To our knowledge, no such efforts have been initiated in the invaded range to date (Turan et al., 2017) and the synthetic production of TTX, either chemosynthetically or through microbial synthesis, is considered to provide a more stable, reliable and cost-effective method than the extraction from harvested pufferfish (Yu, 2007; Lago et al., 2015). A feasibility analysis of such a commercial undertaking is probably warranted. However, any management measure involving the creation of a new market should be carefully implemented as it would introduce conflicting management objectives (i.e. pressure against 'overexploitation' of the species so that the new fishery is sustainable vs. maximum possible removal to minimize adverse impacts of the species – for an example of such a management conundrum see the *Rapana venosa* case in the Black Sea - Todorova, 2012; Janssen et al., 2014). The same applies to measure M3.	Low
	M3. Depuration from TTX with the purpose of human consumption	The depuration of TTX from pufferfish tissues has been demonstrated both in live animals in captivity (Noguchi et al., 2006) and in processed flesh and internal organs	High

		(Hwang & Noguchi, 2007; Anraku et al., 2013) with procedures that can take up to 3 years. Besides the high requirements in space, time and investment of such a scheme, public concern about the toxicity of the species before depuration will most likely make sale/marketing problematic. Moreover, an endogenous source of TTX has been confirmed for a different Tetraodontidae species (*Fugu niphobles*, Matsumura, 1998), urging a re-examination of the TTX acquisition mechanism in pufferfish and possible causing complications for the depuration of live animals.	
	M4. Regional **co-ordination** and policy integration with non-EU countries bordering the Mediterranean where *L. sceleratus* is already present or expected to arrive	This would be important both for monitoring and for population control efforts. See E2 for (sub)regional monitoring plans under the coordination of GFCM.	High
	M6. For the mitigation of impacts: **awareness campaigns** to fishermen and the general public for the toxicity of the species	Such measures have already been implemented throughout the invaded range. It is noteworthy that in EU Member States where the species is already considered invasive (i.e. Cyprus and Greece) no fatal incidents from consumption have been reported and only a small number of severe intoxications is known, restricted to tourists and transient maritime professionals (RA document, Annex VI).	High See https://isea.com.g press-release-toxic-poison alien-species-occurring-mediterranean-sea/?lang=

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