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Risk analysis report of non-native organisms in Belgium - American bullfrog *Lithobates catesbeianus* (Shaw)

Tim Adriaens, Sander Devisscher and Gerald Louette

inbo
Instituut voor
Natuur- en Bosonderzoek

Auteurs:
Tim Adriaens, Sander Devisscher en Gerald Louette

Instituut voor Natuur- en Bosonderzoek
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Vestiging:
INBO Brussel
Kliniekstraat 25, 1070 Brussel
www.inbo.be

e-mail:
tim.adriaens@inbo.be

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Risk analysis report of non-native organisms in Belgium

Risk analysis of American bullfrog *Lithobates catesbeianus* (Shaw)

Developed by : Tim Adriaens, Sander Devisscher, Gerald Louette (INBO)

Reviewed by : Robert Jooris (Hyla vzw), An Martel (Ghent University Department of Pathology, bacteriology and poultry diseases), Frank Pasmans (Ghent University Department of Pathology, bacteriology and poultry diseases), Riccardo Scalera (IUCN SSC Invasive Species Specialist Group)

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Etienne Branquart (Cellule Espèces Invasives, Service Public de Wallonie) developed the risk analysis template that was used for this exercise.

The general process of drafting, reviewing and approval of the risk analysis for selected invasive alien species in Belgium was attended by a steering committee, chaired by the Federal Public Service Health, Food chain safety and Environment. RBINS/KBIN was contracted by the Federal Public Service Health, Food chain safety and Environment to perform PRA's for a batch of species. ULg was contracted by Service Public de Wallonie to perform PRA's for a selection of species. INBO and DEMNA performed risk analysis for a number of species as in-kind contribution. Steering committee members were:

Tim Adriaens	Research Institute for Nature and Forest (INBO)
Olivier Beck	Brussels Environment (BIM)
Roseline Beudels-Jamar	Royal Belgian Institute of Natural Sciences (RBINS/KBIN)
Etienne Branquart	Département de l'Etude du Milieu Naturel et Agricole (DEMNA)
Jim Casaer	Research Institute for Nature and Forest (INBO)
Thibaut Delsinne	Royal Belgian Institute of Natural Sciences (RBINS/KBIN)
Maud Istasse (chair)	Federal Public Service Health, Food chain safety and Environment
René-Marie Lafontaine	Royal Belgian Institute of Natural Sciences (RBINS/KBIN)
Alice Lejeune	Federal Public Service Health, Food chain safety and Environment
Céline Prévot	Département de l'Etude du Milieu Naturel et Agricole (DEMNA)
Henri Robert	Royal Belgian Institute of Natural Sciences (RBINS/KBIN)
Vinciane Schockert	Université de Liège (ULg)
Sonia Vanderhoeven	Belgian Biodiversity Platform (BBPF)
Hans Van Gossum	Agency for Nature and Forest (ANB)
Hugo Verreycken	Research Institute for Nature and Forest (INBO)

Rationale and scope of the Belgian risk analysis scheme

The Convention on Biological Diversity (CBD) emphasises the need for a precautionary approach towards non-native species. It strongly promotes the use of robust and good quality risk assessment to help underpin this approach (COP 6 Decision VI/23). More specifically, when considering trade restrictions for reducing the risk of introduction and spread of a non-native organisms, full and comprehensive risk assessment is required to demonstrate that the proposed measures are adequate and efficient to reduce the risk and that they do not create any disguised barriers to trade. This should be seen in the context of WTO and free trade as a principle in the EU (Baker et al. 2008, Shine et al. 2010, Shrader et al. 2010).

This risk analysis has the specific aim of evaluating whether or not to install trade restrictions for a selection of absent or emerging invasive alien species that may threaten biodiversity in Belgium as a preventive risk management option. It is conducted at the scale of Belgium but results and conclusions could also be relevant for neighbouring areas with similar eco-climatic conditions (e.g. areas included within the Atlantic and the continental biogeographic regions in Europe).

The risk analysis tool that was used here follows a simplified scheme elaborated on the basis of the recommendations provided by the international standard for pest risk analysis for organisms of quarantine concern[1] produced by the secretariat of the International Plant Protection Convention (FAO 2004). This logical scheme adopted in the plant health domain separates the assessment of entry, establishment, spread and impacts. As proposed in the GB non-native species risk assessment scheme, this IPPC standard can be adapted to assess the risk of intentional introductions of non-native species regardless the taxon that may or not be considered as detrimental (Andersen 2004, Baker et al. 2005, Baker et al. 2008, Schrader et al. 2010).

The risk analysis follows a process defined by three stages : (1) the initiation process which involves identifying the organism and its introduction pathways that should be considered for risk analysis in relation to Belgium, (2) the risk assessment stage which includes the categorization of emerging non-native species to determine whether the criteria for a quarantine organism are satisfied and an evaluation of the probability of organism entry, establishment, spread, and of their potential environmental, economic and social consequences and (3) the risk management stage which involves identifying management options for reducing the risks identified at stage 2 to an acceptable level. These are evaluated for efficacy, feasibility and impact in order to select the most appropriate. The risk management section in the current risk analysis should however not been regarded as a full-option management plan, which would require an extra feasibility study including legal, technical and financial considerations. Such thorough study is out of the scope of the produced documents, in which the management is largely limited to identifying needed actions separate from trade restrictions and, where possible, to comment on cost-benefit information if easily available in the literature.

This risk analysis is an advisory document and should be used to help support Belgian decision making. It does not in itself determine government policy, nor does it have any legal status. Neither

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[1] A weed or a pest organism not yet present in the area under assessment, or present but not widely distributed, that is likely to cause economic damages and is proposed for official regulation and control (FAO 2010).

should it reflect stakeholder consensus. Although the document at hand is of public nature, it is important to realise that this risk assessments exercise is carried out by (an) independent expert(s) who produces knowledge-based risk assignments sensu Aven (2011). It was completed using a uniform template to ensure that the full range of issues recognised in international standards was addressed.

To address a number of common misconceptions about non-native species risk assessments, the following points should be noted (after Baker et al. 2008):

- Risk assessments are advisory and therefore part of the suite of information on which policy decisions are based;
- The risk assessment deals with potential negative (ecological, economic, social) impacts. It is not meant to consider positive impacts associated with the introduction or presence of a species, nor is the purpose of this assessment to perform a cost-benefit analysis in that respect. The latter elements though would be elements of consideration for any policy decision;
- Completed risk assessments are not final and absolute. New scientific evidence may prompt a re-evaluation of the risks and/or a change of policy.

Executive summary

PROBABILITY OF ESTABLISHMENT AND SPREAD (EXPOSURE)	
Entry in Belgium	The pathways of introduction in Belgium remain largely undocumented. The risk of escaping American bullfrogs from captivity is currently considered low as compared to other introduction pathways such as deliberate introductions for ornamental purposes and accidental introduction through contaminated fish lots. Natural colonisation from neighbouring countries is considered unlikely.
Establishment capacity	American bullfrog has flexible life-history strategies in favour of successful establishment from only a very limited number of founders. It is a generalist species that can occupy a broad range wetland habitat types and profits from human interference in the landscape. The species has a high reproductive capacity and few natural enemies in Belgium. Belgian climatic variables are well within it physiological requirements and climatic conditions are not limiting successful establishment. Meanwhile, it is well established in Belgium for more than a decade and has invaded areas of conservation concern.
Dispersion capacity	Natural dispersal in American bullfrog can be considerable (> 1km/year). Translocation by humans often complements natural spread.
EFFECT OF ESTABLISHMENT	
Environmental impacts	There is substantial evidence that American bullfrog may negatively affect native amphibians through competition for resources and through direct and indirect predation effects. The effects will be enhanced when species are under pressure from other factors, such as habitat loss, fragmentation, habitat degradation or the presence of invasive fish. Moreover, the bullfrog is a vector of a number of important amphibian diseases that have been implicated in amphibian declines in Europe and throughout the world.
RISK MANAGEMENT	
The relative importance of accidental importation (e.g. through fish stocking) versus natural dispersal or secondary translocations is unknown. Accidental importation of bullfrog larvae hitchhiking in fish stocking lots for angling, is also a pathway to consider. The extent of this phenomenon is however unknown. As there are no reports on farming facilities in Belgium, these pathways are likely to be more important than escapes from rearing facilities. Data on trade of American bullfrog (e.g. in pet shops, on internet) are lacking. In light of these uncertainties, the precautionary principle seems an appropriate way to reduce the risk of both deliberate and accidental importations of American bullfrog in Belgium. The species can easily be overlooked at early stages of invasion. Small, isolated populations can be removed but large interconnected metapopulations are extremely hard to tackle and eradication or control is probably only feasible at very high cost here. Therefore, prevention of further spread, a targeted action plan for existing populations and a dedicated early warning are crucial in tackling this invasive alien in Belgium.	

Samenvatting

WAARSCHIJNLIJKHEID VAN VESTIGING EN VERSPREIDING (BLOOTSTELLING)	
Introductie in België	De introductietrajecten voor stierkikker in België zijn slecht gedocumenteerd. Het risico dat de dieren uit gevangenschap zouden ontsnappen, wordt als laag beschouwd, vergeleken met andere introductiewegen zoals de opzettelijke introductie voor sierdoeleinden en de onopzettelijke introductie door met larven gecontamineerde partijen vis. Natuurlijke kolonisatie vanuit buurlanden wordt als onwaarschijnlijk beschouwd.
Vestigingsvermogen	De stierkikker heeft een flexibele levenswijze die succesvolle vestiging vanuit een beperkt aantal stichters mogelijk maakt. De soort is een generalist die zich kan vestigen in een brede waaier van watergebonden habitats en profiteert van menselijke ingrepen in het landschap. Ze beschikt over een hoge reproductiecapaciteit en heeft in België maar weinig natuurlijke vijanden. Het klimaat in België valt binnen haar fysiologische vereisten en klimaatomstandigheden zijn niet limiterend voor vestiging. De soort is ondertussen al meer dan een decennium ingeburgerd in België en heeft zich in beschermde gebieden gevestigd.
Verspreidingsvermogen	De natuurlijke verbreidingscapaciteit van stierkikker is aanzienlijk aanzienlijk verbreiden (> 1km/jaar). Deze wordt nog aangevuld met secundaire translocaties door de mens.
EFFECTEN VAN DE VESTIGING	
Milieu-impact	Er is overvloedig wetenschappelijk bewijs dat stierkikker andere amfibieën negatief kan beïnvloeden door competitie voor voedsel en ruimte en door predatie. Deze effecten worden groter naarmate soorten nog andere milieudrukken ondervinden, zoals habitatverlies, fragmentatie, habitat degradatie of de aanwezigheid van invasieve vissoorten. Bovendien is stierkikker ook drager van een aantal belangrijke amfibieënziektes.
RISICOBEHEER	
Het relatieve belang van onopzettelijke introductie (vb. door het uitzetten van vis) ten opzichte van natuurlijke dispersie of secundaire translocatie is niet gekend. De onopzettelijke invoer van larven van de stierkikker via het uitzetten van partijen vis voor hengelaars, dient als introductieweg eveneens in aanmerking te worden genomen. Welke omvang dit verschijnsel aanneemt is echter niet bekend. Vermits er geen meldingen zijn van commerciële kwekerijen in België zijn die introductiewegen wellicht belangrijker dan ontsnappingen uit kweek. Gegevens over de handel in stierkikkers (vb. dierenwinkels, internet, ...) ontbreken. Gezien die vele onzekerheden lijkt het hanteren van het voorzorgsprincipe het meest aangewezen om het risico van zowel opzettelijke als onopzettelijke invoer in België te beperken. De soort blijft tijdens de vroege stadia van invasie gemakkelijk onopgemerkt. Kleine, geïsoleerde populaties kunnen worden verwijderd, maar op grote, onderling verbonden metapopulaties kan men moeilijk vat krijgen. Uitroeiing of beheer zijn wellicht enkel mogelijk tegen een hoge kost. Om deze invasieve uitheemse soort in België aan te pakken zijn het voorkomen van verdere verspreiding, het uitwerken van een gericht actieplan voor bestaande populaties en een toegespitst vroegtijdig waarschuwingssysteem van cruciaal belang.	

Résumé

PROBABILITE D'ETABLISSEMENT ET DE DISSEMINATION (EXPOSITION)	
Introduction en Belgique	Les voies d'introduction en Belgique restent largement non documentées. Le risque d'évasion des Grenouilles taureau captives est actuellement considéré comme faible comparativement aux autres voies d'introduction comme les introductions délibérées à des fins ornementales ou les introductions accidentelles par le biais de lots contaminés de poissons dans le cadre de ré-empoissonnement. La colonisation naturelle à partir des pays voisins est considérée peu probable.
Capacité d'établissement	La Grenouille taureau met en place des stratégies de survie flexibles qui favorisent la réussite de son implantation à partir d'un nombre très limité d'individus fondateurs. Il s'agit d'une espèce généraliste qui peut occuper un vaste éventail d'habitats humides et qui tire avantage des perturbations humaines dans l'environnement. Cette espèce démontre une grande capacité reproductive et connaît peu d'ennemis naturels en Belgique. Les conditions climatiques belges cadrent avec ses besoins physiologiques et ne limitent donc pas la probabilité de réussite de ses implantations. Elle est de fait bien établie en Belgique depuis déjà plus d'une décennie maintenant et a envahi des zones sensibles pour la conservation de la nature.
Capacité de dispersion	La vitesse de dispersion naturelle de la Grenouille taureau peut être importante (>1km/an). Son transport par l'homme complète souvent sa dispersion naturelle.
EFFET DE L'ETABLISSEMENT	
Impacts environnementaux	On dispose de preuves substantielles montrant que la Grenouille taureau peut avoir un effet négatif sur les amphibiens indigènes par compétition pour les ressources et par des effets de prédation directe et indirecte. Ces effets sont encore renforcés quand l'espèce indigène est déjà sous pression en raison d'autres facteurs, notamment perte d'habitat, fragmentation, dégradation de l'habitat ou la présence d'espèces invasives de poissons. De plus, la Grenouille taureau est vecteur de plusieurs maladies propres aux amphibiens dont celles responsables de la diminution des amphibiens en Europe et dans le monde entier.
GESTION DES RISQUES	
L'importance de l'importation accidentelle (par l'empoissonnement) en comparaison avec la dispersion naturelle ou le transfert secondaire n'est pas connue. L'importation accidentelle de têtards de Grenouille taureau dans les lots de réempoissonnement pour la pêche à la ligne constitue une voie potentielle d'introduction à prendre en compte. L'importance de ce phénomène est toutefois inconnue. Etant donné qu'on ne dispose pas d'information sur l'existence d'installations d'élevage en Belgique, les voies d'introduction expliquées ci-dessus sont sans doute plus importantes que les échappés d'élevage. On ne dispose pas de données sur le commerce de la Grenouille taureau (p. ex. dans les animaleries ou sur internet). A la lumière de ces incertitudes, l'application du principe de précaution semble le plus approprié pour réduire le risque d'importation à la fois délibérée et accidentelle. Cette espèce peut facilement passer inaperçue aux stades précoces d'invasion. De petites populations isolées peuvent être éradiquées mais les grandes populations interconnectées sont extrêmement difficiles à traiter et leur éradication ou leur contrôle n'est probablement possible qu'à des coûts très élevés. Pour cette raison, la prévention de toute dispersion future, un plan d'action ciblé pour gérer les populations existantes et un système d'avertissement précoce revêtent une importance cruciale dans le traitement de cette espèce.	

STAGE 1: INITIATION

Precise the identity of the invasive organism (scientific name, synonyms and common names in Dutch, English, French and German), its taxonomic position and a short morphological description. Present its distribution and pathways of quarantine concern that should be considered for risk analysis in Belgium. A short morphological description can be added if relevant. Specify also the reason(s) why a risk analysis is needed (the emergency of a new invasive organism in Belgium and neighbouring areas, the reporting of higher damages caused by a non-native organism in Belgium than in its area of origin, or request made to import a new non-native organism in the Belgium).

1.1 ORGANISM IDENTITY

Scientific name : *Lithobates catesbeianus* (Shaw 1802)
Synonyms: *Rana catesbeiana* Shaw 1802
Rana taurina Cuvier 1817
Rana mugiens Merrem 1820
Rana scapularis Harlan 1826
Rana conspersa LeConte 1855
Common names : Amerikaanse stierkikker, Amerikaanse brulkikker, rundkikker (Dutch)
bullfrog, North American bullfrog (English)
grenouille taureau, ouaouaron, grenouille mugissante (French)
(Nord)amerikanischer Ochsenfrosch (German)
Rana toro (Spanish, Italian)
Taxonomic position: Chordata (Phylum) > Amphibia (Class) > Anura (Order) > Ranidae (Family)

The American bullfrog *Lithobates catesbeianus* (Shaw 1802) (syn. *Rana catesbeiana*) belongs to the family of the True frogs (Ranidae), an almost cosmopolitan frog family (except Australia and southern South America) that comprises some 350 species worldwide (Frost, 2011; Frost et al., 2006). The name change *R. catesbeiana* to *L. catesbeianus* is the result of new insights in the phylogeny of the family. For a detailed discussion of systematics, nomenclature, taxonomy and phylogeny, we refer to the online database Amphibian Species of the World (Frost, 2011). The genus *Lithobates* Fitzinger 1843, a sister taxon to *Rana* Linnaeus 1758, counts about fifty species worldwide, distributed throughout North, Central and South America (Frost, 2011).

1.2 SHORT DESCRIPTION

American bullfrog is in all life stages (larva, metamorph, adult) a large amphibian. Adults can reach up to 22 cm snout-vent length (SVL) and can weigh more than 500 grams (Thomas & Wogan, 1999). They have a robust body with a wide, flat head and smooth skin with little wrinkles, warts or spikes. Females are on average slightly larger than males, which can also be recognised by the yellow throat colour (Figure 1) and the presence of black nuptial pads on the thumbs during the mating season (Stumpel & Strijbosch, 2006). Tadpoles range between 4-17 cm, metamorphs 7-10 cm. Juvenile, metamorphosed bullfrogs in Flanders were 4.5 cm SVL on average and weighed 12 g (Jooris, 2005). The heaviest adult bullfrog recorded in Belgium weighed 560 grams (Jooris, 2005). However, because marsh frogs *Pelophylax ridibundus* can overlap in size, the size alone is not conclusive for identification. Adult bullfrogs, besides their size, distinguish themselves from resembling native species by the presence of a large tympanum (as large or larger than the eye diameter) and by the lack of dorsolateral glands (Jooris, 2012; Van Diepenbeek et al., 2010). Adult bullfrogs have a clear skin fold from around the ear to the base of the forelegs. Unlike marsh frogs and other green frogs, they lack a green/yellow dorsal stripe (Nöllert et al., 2001).

Figure 1: Adult male American bullfrog (© Jan Van Der Voort).

Larvae of green frogs *Pelophylax* spp. are similar to bullfrog larvae and can prove difficult to distinguish. Bullfrog larvae show diverse colour patterns but are usually brown to olive-green on the back and white to yellowish on the belly (Figure 2). They are mostly speckled with brown or black dots over the entire body (Jooris, 2005). Larvae of green frogs are usually between 4-8 cm (Günther, 1990), but it is not uncommon for them, in their second year, to reach a length of more than 10 cm, whereas bullfrog larvae reach 15-18 cm. Newly hatched bullfrog larvae are about the size of larvae of native frogs and toads. At this stage, the presence of small dark brown to black spots is an important diagnostic feature.

Figure 2: American bullfrog larvae in different life stages. The upright animal is a L2 larva (developed hind legs, 80-170mm), sitting animal is a pre-metamorph M1 larva (© Domin Dalessi).

American bullfrogs could possibly be confused with the similar pig frog (syn. southern bullfrog) *Lithobates grylio* (Stejneger, 1901), a frog species from the extreme southeast of the U.S. named after the sound produced by the males during the mating season (Conant & Collins, 1998). The two congeners are sympatric and look alike (Conant and Collins, 1998) which makes accidental import of pig frog a possibility. Besides the grunting sound of the males, pig frogs can be distinguished by a more pointed snout, fins nearly reaching the toe tips (in bullfrog the longest toes clearly protrudes) and remarkably thick, dark spots and stripes on the back of the thighs (bullfrogs have many small bright spots) (Conant and Collins, 1998).

Many reports of adult bullfrogs actually concern wrongly determined green frogs. Due to their size (up to 13 cm) and noisy character, confusion is most likely to occur with oversized marsh frogs. The vocalisation of this species is however completely different (a typical *kek-kek-kek* sound) than the mating *whrumm-whrumm* ("more rum") sound, which is produced by the male with the single vocal sac and is described as the sound of a bull or recalling the sound of a bittern (*Botauris stellaris*). Apart from the mating call, bullfrogs also have a unique call, usually produced by juvenile and subadult frogs and consisting of a thin, shrill *iep* sound. It is produced when the frogs are disturbed and jump into the water. The hearing of this cry is an important clue to the presence of bullfrog when performing inventories from the shoreline (Jooris, 2002b).

1.3 ORGANISM DISTRIBUTION

Native range

Bullfrogs are native to eastern North America, with the Rocky mountains providing the western boundary of their natural range (Bury & Whelan, 1984). The natural range spans a wide latitude and extends north to Canada (Nova Scotia, New Brunswick, southern Quebec and southern Ontario) and south to central Florida and north-eastern Mexico. This vast natural range is reminiscent to the species flexible life history and broad climatic and ecological amplitude, which contributes to its success as an invasive alien (D'Amore, 2012). Bullfrogs occupy a wide range of wetland habitats like ponds, lakes, swamps, bogs, marshes, slowly flowing rivers and streams. They often show a preference for highly artificial and modified habitats, such as millponds, livestock grazing ponds, reservoirs, irrigation ponds and ditches. As a consequence, the establishment of bullfrogs may be favoured by human driven habitat modification, such as changes in hydrology from seasonal to permanent water, removal of emergent vegetation and elevation of water temperatures (D'Amore, 2012; D'Amore et al., 2010; EEA, 2012). Although they can occupy seasonal pools (Gahl et al., 2009), they are mostly relying on permanent water for breeding (Lougheed & Taylor, 2010). In Hawaii, where the species was introduced in the 19th century, the species is also found in brackish pools (Orchard, 2010).

Introduced range

Belgium: The species is established in the Atlantic region in Belgium since the end of the nineties, with first observations in Flanders in 1996 (Jooris, 2002b) and Wallonia in 1992 (De Wavrin et al., 2007; Martin, 2009; Martin et al., 2010).

Europe: Based on genetic analysis, in Europe, at least six independent introductions of different genetic make-up from the native range can be identified (Ficetola et al., 2008a). In chronological order, these are Italy, France, Germany, Belgium, Greece and the UK. These introductions were followed by secondary translocations of the species within the European Union (Ficetola et al., 2007a). The first introductions in Europe occurred in Italy in 1932 (Lanza & Ferri, 1997) and in France in 1968 (Detaint & Coic, 2006), but 60 % of European introductions occurred during the 1980s and 1990s (Ficetola et al., 2007a). Genetic variation in European bullfrog populations was strongly reduced compared to the inferred source populations indicating a very strong bottleneck during colonization due to a limited number of founders (Ficetola et al., 2008a; Funk et al., 2010). The French, Italian, German, and UK populations probably originated from the western part of the native range. The origin of Belgian and Greek populations is still unresolved (Ficetola et al., 2008a). At current, following at least 25 independent introductions, the species is reported in eight countries (Italy, France, Germany, Belgium, Greece, The Netherlands, Spain and Great Britain) (Ficetola et al., 2007a; Ficetola et al., 2007b; Lanza & Ferri, 1997). It occurs in different bio-geographical regions

(Atlantic, Continental, Mediterranean) which illustrates its broad climatic and environmental amplitude (Figure 3). For some countries (Denmark, Croatia), the information on bullfrog occurrence needs to be checked. Reproducing populations are, however, only known for Italy, France, Belgium, The Netherlands and Greece (Crete). Eradication or control programmes have been carried out in Belgium (Devisscher et al., 2012; Louette et al., 2012c), France (Berroneau et al., 2008; Coïc & Detaint, 2001; Detaint & Coic, 2006), Germany (Laufer & Waitzmann, 2002; Thiesmeier et al., 1994), The Netherlands (Creemers, 2011; Crombaghs, 2012; Goverse et al., 2012) and the United Kingdom (website non-native species secretariat, (Ficetola et al., 2007a) (Figure 3).

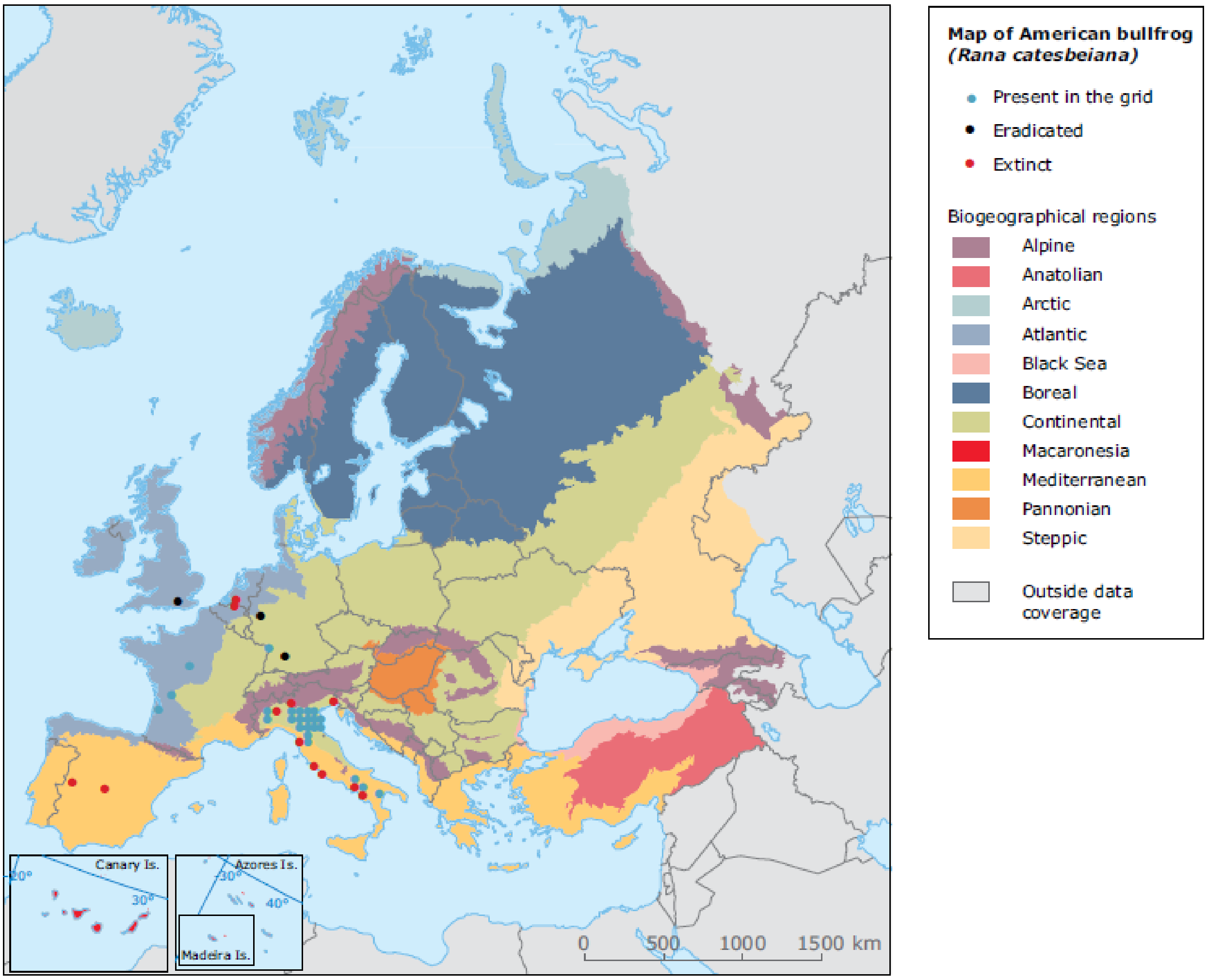


Figure 3: Distribution of American bullfrog in Europa with a distinction between eradicated and extinct populations (EEA, 2012). The map is based on (Ficetola et al., 2007a). For Belgium and The Netherlands, the map shows some gaps (see Figure 6, Figure 7 and Figure 8). Also the island population on Crete is not shown on this map.

<u>Other continents</u>: Bullfrogs have been reported in about 40 countries on all continents, except Antarctica and Africa (Santos-Barrera et al., 2011) (Figure 4). In the US, the species was introduced west of the Rocky Mountains in the late 1800s (Jennings & Hayes, 1985). At current, the introduced range of the species includes many countries in Central (Hawaii, western and southern Mexico and the Caribbean) and South America (Argentina, Paraguay, Peru, Guyana, Brazil, Chile, Colombia, Ecuador, Venezuela), Europe (see higher), Oceania and Asia (Israel, Russia, Malaysia, Philippines, Indonesia including Java and Bali, Japan, China, Taiwan, Korea and Thailand) (Adams & Pearl, 2007;

Ficetola et al., 2010). A more detailed account of invasion history and native and introduced range of bullfrogs can be found in (Devisscher et al., 2012).

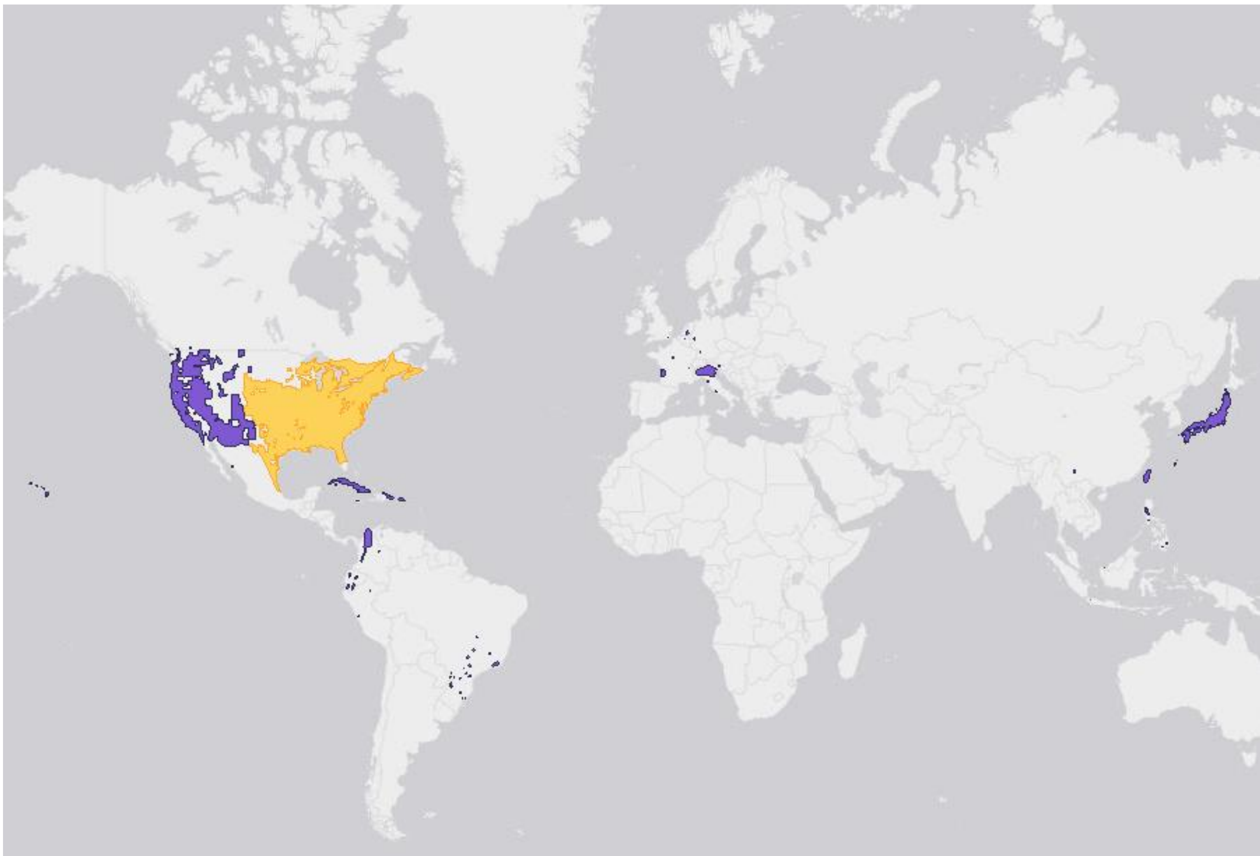


Figure 4: Worldwide distribution of native (yellow) and introduced (blue) populations of American bullfrog based on data from the Global Amphibian Assessment (http://maps.iucnredlist.org) (Santos-Barrera et al., 2011).

1.4 REASONS FOR PERFORMING RISK ANALYSIS

Due to its broad global distribution and widespread effects on native biodiversity through various impact mechanisms, the American bullfrog is listed as one of the top 100 most invasive alien species in the world by the IUCN (Lowe et al., 2000). The species is suspected to cause substantial ecological damage around large parts of the globe, exerting an additional pressure to already declining amphibian populations (Adams & Pearl, 2007). Negative impacts on native biota entail competition, predation and the transmission of pathogens (D'Amore, 2012). Bullfrogs are considered a major threat to biodiversity in general, and amphibians in particular, since they are highly competitive and can act as structuring predators in aquatic ecosystems. Moreover, the species is a healthy carrier of pathogens, such as fungi and viruses, known to infect other amphibians. Subsequently, in Europe, it is listed on the SEBI worst list of invasive non-native species with high impact on biodiversity (EEA, 2007), as well as the DAISIE list of the worst invasive aliens in Europe (DAISIE, 2009). For the same reasons, the species is included in the appendix to the Bern Convention recommendation n° 77 (1999) as a species which has proved to be a strong threat to biological diversity and for which eradication is strongly recommended. Moreover, the EU Wildlife Trade Regulation (338/97) Appendix B prohibits import of the species into the EU since December 1997.

STAGE 2: RISK ASSESSMENT

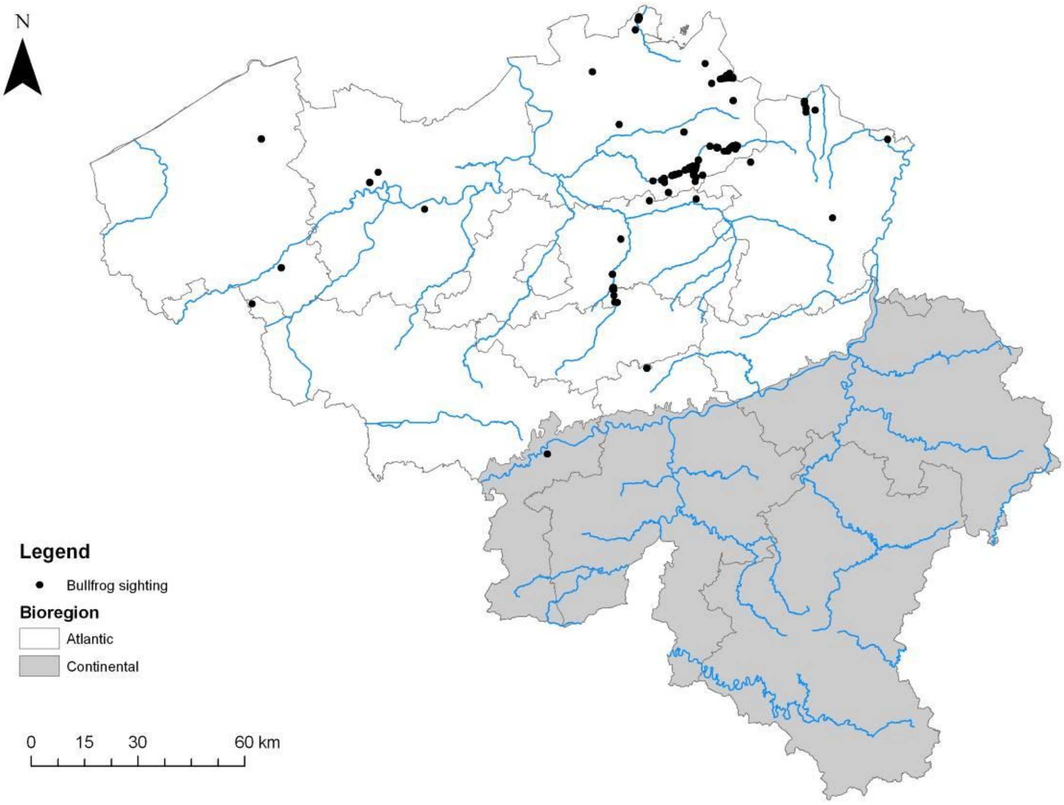
2.1 PROBABILITY OF ESTABLISHMENT AND SPREAD (EXPOSURE)

Evidence should be available to support the conclusion that the non-native organism could enter, become established in the wild and spread in Belgium and neighbouring areas. An analysis of each associated pathways from its origin to its establishment in Belgium is required. Organisms intentionally imported maybe maintained in a number of intended sites for an indeterminate period. In this specific case, the risk may arise because of the probability to spread and establish in unintended habitats nearby intended introduction sites.

2.1.1 Present status in Belgium

Specify if the species already occurs in Belgium and if it makes self-sustaining populations in the wild (establishment). Give detail about species abundance and distribution within Belgium when establishment is confirmed together with the size of area suitable for further spread within Belgium.

The species is established in the Atlantic region in Belgium since the end of the nineties (Figure 5), with first observations in Flanders in 1996 (Jooris, 2002b) and Wallonia in 1992 (De Wavrin et al., 2007; Martin, 2009).



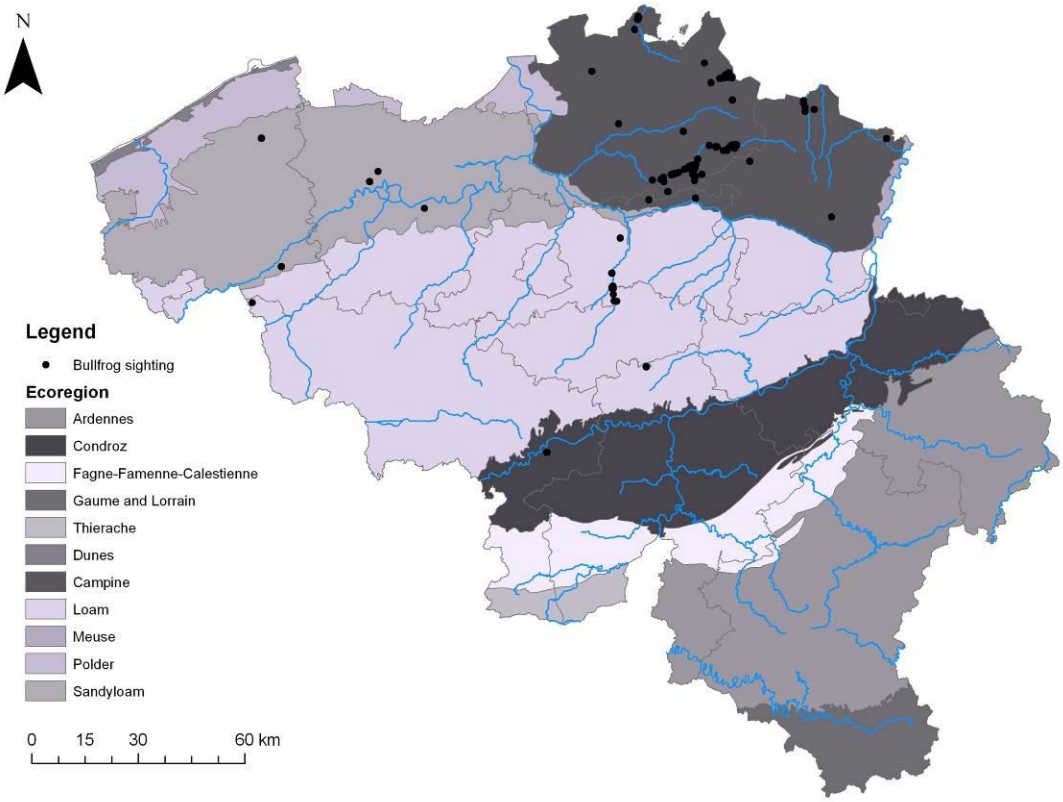


Figure 5: Observations of American bullfrog in Belgium (1992-2012), showing bio-geographical regions (upper) and Belgian ecoregions (lower). Populations and loose observations are lumped. Flemish data: Hyla, working group of Natuurpunt Studie and INBO (Invexo). Walloon data inferred from (De Wavrin et al., 2007; Martin, 2009).

In Flanders, American bullfrogs have been observed in all provinces (Jooris, 2001; Jooris, 2005). In Western and Eastern Flanders, only solitary individuals have been observed. The first proof of reproduction in Flanders dates back to 2001 at several places in the Grote Nete Valley (Jooris, 2002a; Jooris, 2002b; Jooris, 2005). Most of the reproducing populations now occur in the province of Antwerp (Figure 6). The largest stronghold of American bullfrog is a big (meta)population in the valley of the Grote Nete between Westerlo and Balen (Adriaens et al., 2010; Jooris, 2002b). Here, bullfrogs thrive in a complex of several hundreds of - largely private - ponds used for recreational fishing and gardening. These ponds are mostly nutrient rich, with low vegetation structure and high abundance of non-native fish species such as pumpkinseed sunfish (*Lepomis gibbosus*) and topmouth gudgeon (*Pseudorasbora parva*) which provides an ideal habitat for bullfrogs. These fish species are well known predators of macro-invertebrate and zooplankton communities in aquatic ecosystems (e.g. Anseeuw, 2011). Their presence can thereby both reduce the predation pressure by macro-invertebrates (e.g. Dytiscidae, Aeshnidae) on tadpoles (predation release) and increase the food availability for bullfrog larvae through an increase in phytoplankton (facilitation) (Adams & Pearl, 2007; Adams et al., 2003). Furthermore, isolated populations with a few contaminated ponds were found in the Mark valley (Hoogstraten, northern Antwerp) and the Wamp valley (Arendonk, Antwerp), the Dommel (Lommel, Limburg) and the valley of the Dyle in Sint-Agatha-Rode in the nature reserve Grootbroek (Flemish Brabant). Based on depletion and capture-mark-recapture, the number of larvae in ponds at Arendonk, Balen and Hoogstraten varied between 950 and 120.804 specimens per ha (Louette et al., 2012b; Louette et al., 2012c) and an estimated adult density of one individual per ten meter shoreline (Louette et al., subm.). Detailed accounts of invasion history and distribution per province can be found in (Devisscher et al., 2012).

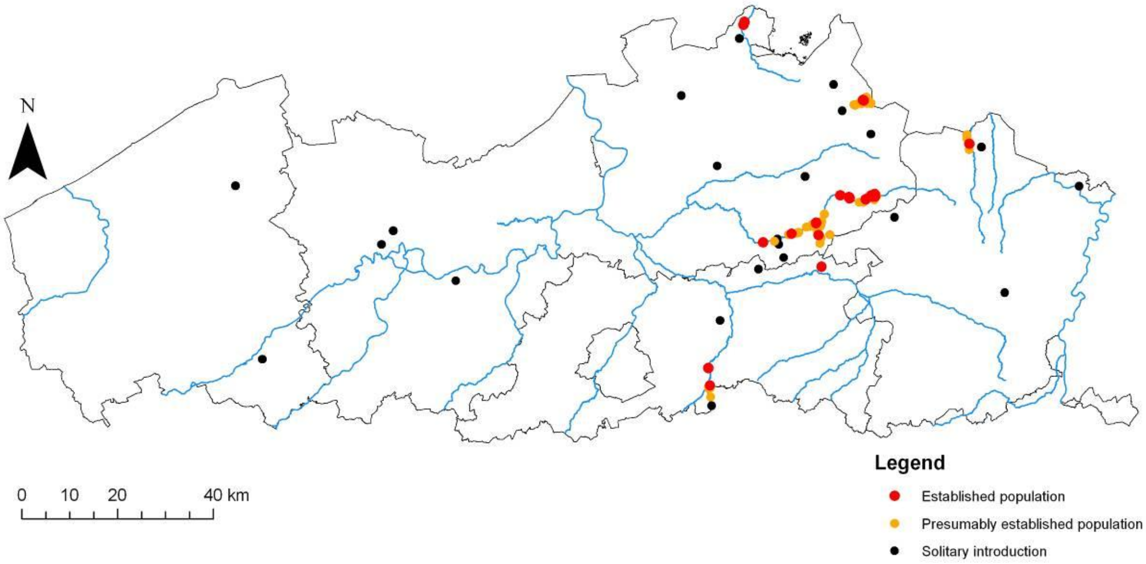


Figure 6: Distribution of American bullfrog in Flanders with a distinction between reproducing populations and observations of solitary individuals (data: Hyla, amphibian and reptile working group of Natuurpunt Studie). Established populations = confirmed observations of larvae; presumably established populations = reports of bullfrog presence within 2.5km (annual dispersal range of American bullfrog) of established population.

In Wallonia, American bullfrog is not yet widely spread and appears to be in an early invasion stage (Martin, 2009; Martin et al., 2009). The species was first observed in 1992 at Jamioulx (Charleroi). Calling males were reported for several years here but seem to have disappeared (De Wavrin et al., 2007). This was also the first observation for Belgium. Between 1997 and 2001, a single specimen was reported in Grand-Leez on the border between Walloon Brabant and Namur. From 1999 onward, several new observations followed rapidly: Nil-Saint-Vincent (two adults and one juvenile, disappeared in 1999), Grez-Doiceau (1999, one adult, disappeared in 2001), Chaumont-Gistoux (2000), Wépion (2004, two animals were killed) and a single calling male in a pool in Charleroi in the neighbourhood of Ransart (2005-2006) (De Wavrin et al., 2007; Martin, 2009; Martin et al., 2009) (Figure 7). In 2000 reproduction was suspected in Walloon Brabant (de Wavrin, 2000), but at current the only known reproducing populations are located in the Dyle valley (Sint-Agatha-Rhode), upstream the border of Flemish Brabant and Wallonia, and at Ransart (Charleroi). In 2006 the Dyle valley population seemed to extend along a valley stretch of four kilometres between Sint-Agatha-Rhode, Ottenbourg and Florival (De Wavrin et al., 2007). A 2008 inventory, however, did not yield additional sightings in the valley besides the known population in Grootbroek nature reserve (about 10 calling males and presence of tapoles) and one calling male at Etang du Grand Pré (Pécrot) at about 1 km from Grootbroek (Martin, 2009). In 2012, the supposed source lake in Sint-Agatha-Rhode (Flemish side) was checked by means of fyke netting by the Agency for Nature and Forest. This exploratory sampling yielded no evidence of bullfrog presence (pers. comm. H. van Gossum). According to the site owner, bullfrog have been present in Ransart (Charleroi) for more than 15 years (Martin, 2009). The surroundings of this site were also checked in 2008 and yielded one adult at a site nearby (< 500m), indicating the population in this urban context remains localised and is currently not spreading. At the source population in Ransart about 20 males were heard calling in 2008 and also tadpoles were present (Martin, 2009).

At current, no observations have been reported for Brussels Capital Region.

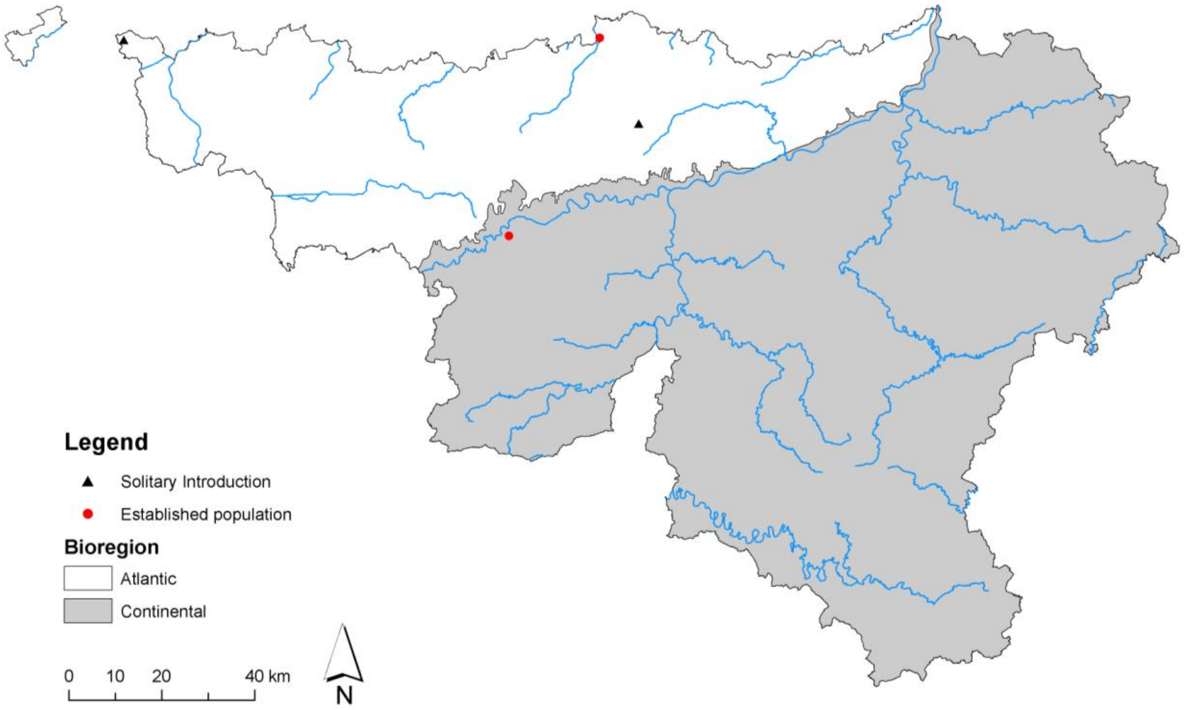


Figure 7: Distribution of American bullfrog in Wallonia since 1985, with established populations as red dots (based on De Wavrin et al. (2007) and Martin (2009).

2.1.2 Present status in neighbouring countries

Mention here the status of the non-native organism in the neighbouring countries.

The Netherlands: In 1991 it was confirmed that a bullfrog population successfully reproduced in a garden pond (250 m^2) in Breda since 1989 (Stumpel, 1992). This population was the result of the introduction of five bullfrog larvae from Belgium in 1986 by the pond owner. An eradication campaign was launched in 1990 and by 1991-1992 this population was wiped out (Lever, 2003; Veenvliet & Veenvliet, 2002). Over the period 1971-1979, successful reproduction has been registered three times, with reproducing animals present in open-air terrariums in Limburg until 2003, illustrating successful bullfrog survival in the Netherlands (Figure 8) (Spitzen-Van der Sluijs et al., 2010; Veenvliet & Veenvliet, 2002). Besides the abovementioned populations, there are also quite a lot of reports of solitary individuals at different locations that have either been caught or have disappeared (Veenvliet, 1996). In 2009 for instance, a single adult male was reported in Sint-Oedenrode (Noord-Brabant), near the Belgian-Netherlands trans-boundary river valley of the Dommel. This animal was shot (Spitzen-van der Sluijs & Zollinger, 2010b). In 2002 a reliable sighting exists in an Amsterdam garden pond, in 2009 a single bullfrog was captured in Limburg. In September 2010 a reproducing population was discovered in two breeding ponds in Baarlo (Limburg), north of Roermond and east of Eindhoven, as a direct result of increased media coverage following the launch of an early warning initiative in the framework of an EU Interreg project (www.invexo.be) for bullfrog in the border region with Belgium. Based on interviews with locals, bullfrog seems to have been present (and unnoticed) for more than ten years in the area (pers. comm. R. Creemers). After a thorough inventory was performed (Creemers, 2011), the populations in two breeding ponds were promptly removed in 2011-2012 through a combination of fencing, seine netting, fyke netting, electrofishing and drainage of one pond. Actions were properly documented and a nearby site was screened for bullfrog presence with environmental DNA techniques (Crombaghs, 2012; Goverse et al., 2012).

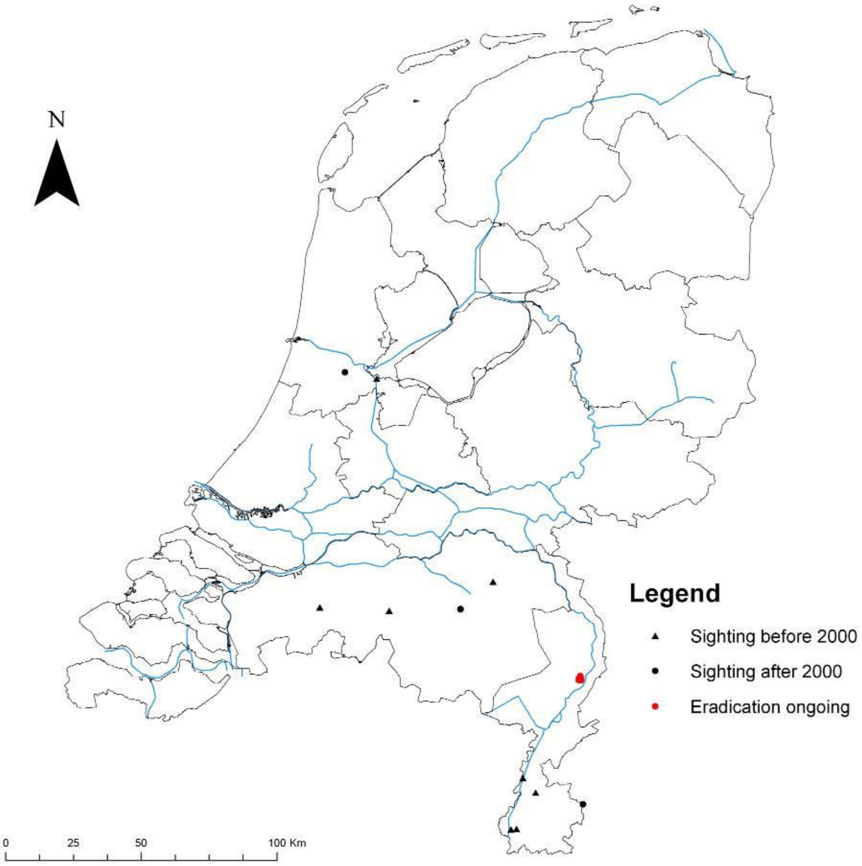


Figure 8: Sightings of American bullfrog in The Netherlands (1986-2012). In red is the location of recent eradication campaign near Baarlo (details in text). After data from RAVON.

<u>Luxemburg:</u> no occurrence of American bullfrog reported.

<u>France:</u> The first release of American bullfrog in France near Bordeaux (Gironde) in 1968, 30 years after the introduction in Italy (Berroneau et al, 2008; Detaint & COIC, 2006). These animals have a United States origin and most probably formed the basis of the current populations in south western France (Ficetola et al., 2007a). A second introduction was performed in the 1980s when a dozen frogs were introduced in a pool in the Gironde. At current, three reproducing populations are known in France in the southwest (Gironde and Dordogne) and central France (Sologne) (Berroneau et al., 2008; Coïc & Detaint, 2001; Detaint & Coic, 2006). Ficetola et al. (2007) performed extensive calling surveys in the southwest area. Bullfrogs were found over a very large area (> 2000 km^2), often discontinuous and too far for spontaneous dispersal of the species, suggesting human-mediated

translocations within the area. The species is established in several distinct nuclei: north of the Dordogne, in three areas (around Piégut, near St. Saud-Lacoussière and near Thiviers), southeast of the Bay of Arcachon and between Libourne and the estuary of the Gironde (Berroneau et al., 2008). The populations in Dordogne and Gironde are subject to an eradication campaign, using direct capture and trapping of both adults and tadpoles, and education of local people to the problems caused by biological invasions (Berroneau et al., 2008; Detaint & Coic, 2006). Furthermore, the literature cites observations in Bois de Bologne (Paris), Parc de Beaujardin (Tours) and the Somme region in 2002 (Veenvliet & Veenvliet, 2002). There may have been reproduction here but no populations have been reconfirmed so far.

Germany: According to Veenvliet & Veenvliet (2002) there are five sites in Germany where bullfrog has or had established. Bullfrogs were first observed in the country in 1934. The first population originated from an escape from an enclosed fishpond near Celle (Lower Saxony, central Germany). Tadpoles and metamorphs were found here in 1935. Between 1935 and 1941 this population was successfully eradicated (Veenvliet & Veenvliet, 2002). In 1990 several bullfrog larvae were released in a garden pond near Böblingen (Stuttgart, Baden-Württemberg) (Laufer & Waitzmann, 2002). Two years later successful reproduction was noted. In 2002, this was eradicated through electrofishing with fencing (Laufer & Waitzmann, 2002; Thiesmeier et al., 1994). In 1993 some adults were observed in northern Germany near Kiel (Schleswig-Holstein). This population was deliberately introduced by a local fish farmer who wanted to bring down the population of common spadefoot (*Pelobates fuscus*) in the pond. No reproduction occurred at this location and the animals that were present died over winter in 1996. Anno 1995 a population with 5-8 calling males and hundreds of newly metamorphosed juveniles was discovered in Meckenheim near Bonn, where according to the locals it had been present for five years already (North Rhine-Westphalia). In 2002 an eradication campaign was launched here involving fencing and several drainage actions and by 2003 it was partially eradicated (Laufer & Waitzmann, 2002). In November 2000 members of a Sports Fishing Association discovered bullfrog larvae near Karlsruhe (Baden-Württemberg). From July to October 2001 about 40 (sub)adults were detected at ten different lakes and ponds, and larvae were found at least at five locations (Laufer & Waitzmann, 2002). This population possibly originates from bullfrogs that were released into the wild in 1992 when a local pet store closed. Because of the private nature of the ponds this large (meta)population had probably remained under the radar for years providing opportunity for expansion into various waters within a 5.5 km radius, notably old sand pits and meanders of the Rhine. Based on measurements and classifying the size of the animals caught, it was assumed that reproduction had taken place on a regular basis in the last five years. In 2002 a management campaign was started. Five infested ponds were pumped out twice, with the help of 20 volunteers and the local fire department. Adults and tadpoles were removed. In addition, these ponds were electronically fished twice (Weizmann, 2002). Costs for these measures were as follows: 20 volunteers, working occasionally over the course of a year, roughly represent the equivalent of one full-time employee, hence € 50,000. The cost for pumping and electrofishing larvae was € 500 and € 1,200 per day, respectively. This predicts an annual cost of € 53,000 per pond per year, thus for five ponds, € 270,000 (ranging € 260,000 - € 520,000) (EEA, 2012; Reinhardt et al., 2003). Despite the massive capture of larvae and juvenile frogs by electrofishing, hand netting and night lighting, bullfrogs were still present in the Upper Rhine area by 2007 (Ficetola et al., 2007a).

Great-Britain: A bullfrog population existed in Surrey (south east England) around 1900 (Lanza & Ferri, 1997) but has now disappeared. New introductions originating from animal trade took place in 1996 and lead to populations in 1999 in a series of (fish) ponds in a stream valley, surrounded by mixed farmland and woodland at Edenbridge on the Kent-Sussex border (south east England) (Langton et al., 2011). Some of the juveniles that were euthanized in 2004 tested positive for fungus infection with *Batrachochytrium dendrobatidis* (Cunningham et al., 2005). Meanwhile, the vicinity of the original breeding sites (two fish ponds) has been subject to detailed monitoring and the populations have been targeted by an eradication programme involving fencing, pitfall trapping, excavation of sediment and light-assisted hand captures at dusk (Banks et al., 2000; Langton et al., 2011). Around 12,000 adult and tadpole bullfrogs were removed from the site between 1999 and 2004 (Cunningham et al., 2005). The implementation of these measures was estimated at a foreseen cost of around 100,000 £ across seven ponds (EEA, 2012). The population is believed to have disappeared (website GB Non-Native Species Secretariat).

2.1.3 Introduction in Belgium

Specify what are the potential international introduction pathways mediated by humans, the frequency of introduction and the number of individuals that are likely to be released in Europe and in Belgium. Consider the potential for natural colonisation from neighbouring areas where the species is established and compare with the risk of introduction by the human-mediated pathways. In case of plant or animal species kept in captivity, assess risk for organism escape to the wild (unintended habitats).

A number of pathways have worldwide been identified for the introduction of American bullfrogs into the wild (Bomford et al., 2005; Bury & Whelan, 1984; EEA, 2012): unintentional escapes from breeding facilities where the species was farmed for human consumption or for trade in aquaculture (Jennings & Hayes, 1985), escapes from garden ponds where it was introduced as an ornamental species, accidental introduction and spread as a stowaway in fish stockings (Hammerson, 1982), intentional releases aimed at establishing wild populations for hunting or harvesting purposes, trade for educational and scientific use and intentional releases for biological control of arthropod pest species (e.g. on Hawaii). In Europe the most important pathways for introduced populations were accidental escapes from commercial farming facilities (5), animal trade and escape of pets (6), deliberate introduction (5) (Ficetola et al., 2007a). In the majority of cases information about the causes and the actual pathway of introduction is unknown.

Its large size makes American bullfrog a good candidate for frog leg production. Internationally, several frog farms were established (Spain, Italy). However, most of the bullfrog farms in Europe are now closed due to both competition with Asian farms and the 1997 ban of the importation of live bullfrogs from outside the European Union. Only five introductions in Europe were performed as attempts at commercial farming (Ficetola et al., 2007a). This is in contrast to observations in other continents, where the bullfrog is usually introduced for the production of food (Lever, 2003). We could find no evidence that facilities for commercial bullfrog farming ever existed in Belgium, so this pathway seems at current of little relevance for Belgium. At current, there is no information available on the keeping of bullfrogs in rearing facilities or private terraria in Belgium. Provided it is a safe assumption that the 1997 regulation has been effective in preventing such facilities to exist, the risk of escaping American bullfrogs from captivity is considered low as compared to other introduction pathways. Another plausible pathway for the introduction and spread of the bullfrog in Belgium is the transportation of contaminated fish lots for aquaculture purposes. Besides occasional adult animals, also eggs and larvae could be transported this way. No evidence has been found that this has occurred in Belgium however, but this has been documented elsewhere in Europe (Veenvliet & Veenvliet, 2002) and has also been suggested for the USA (Hammerson, 1982). In view of the presence of large populations of bullfrog in France, Italy and Germany, this might at current be one of the most important pathways for secondary translocation of American bullfrog within Europe. The fact that some of the bullfrog populations in Belgium occur in the vicinity of aquaculture sites and in ponds used for recreational fishing with regular fish stocking events might indicate the importance of this pathway is largely underestimated.

ENTRY IN BELGIUM

The pathways of introduction in Belgium remain largely undocumented. The risk of escaping American bullfrogs from captivity is currently considered low as compared to other introduction pathways such as deliberate introductions for ornamental purposes and accidental introduction through contaminated fish lots. Natural colonisation from neighbouring countries is considered unlikely.

2.1.4 Establishment capacity and endangered area

Provide a short description of life-history and reproductive traits of the organism that should be compared with those of their closest native relatives (A). Specify which are the optimal and limiting climatic (B), habitat (C) and food (D) requirements for organism survival, growth and reproduction both in its native and introduced ranges. When present in Belgium, specify agents (predators, parasites, diseases, etc.) that are likely to control population development (E). For species absent from Belgium, identify the probability for future establishment (F) and the area most suitable for species establishment (endangered area) (G) depending if climatic, habitat and food conditions found in Belgium are considered as optimal, suboptimal or inadequate for the establishment of a viable population. The endangered area may be the whole country or part of it where ecological factors favour the establishment of the organism (consider the spatial distribution of preferred habitats). For non-native species already established, mention if they are well adapted to the eco-climatic conditions found in Belgium (F), where they easily form self-sustaining populations, and which areas in Belgium are still available for future colonisation (G).

A/ Life-cycle and reproduction

A number of reproductive life history traits contribute to the success of *L. catesbeianus* as an invasive alien species, such as continuous gonadal development, a long reproductive period and a very high fecundity (Howard, 1978). A female can produce up to two clutches of 1000-25,000 eggs per year up to one quarter of her body mass (Adams & Pearl, 2007), in some extreme cases more than 30,000 eggs (Bury & Whelan, 1984). This is considerably more than the smaller native amphibian species such as common frog (*Rana temporaria*), common toad (*Bufo bufo*) or green frogs (Günther, 1990). Bullfrogs show, unlike other amphibians, no ovarian rest, which enables the female to produce enough eggs to develop a second - usually smaller - clutch. The larvae of the second clutch overwinter, even in the warmest regions (Berger & Uzzel, 1980). However, there is no conclusive evidence that bullfrogs in Belgium can produce a second egg clutch. Egg masses consist of floating, gelatinous "surface films" that can form white, foaming masses on or just below the water surface. These are usually placed between plants to prevent drift. The masses consists of thousands of eggs, black brown on top and pale on the underside, and would sink to the bottom after ± 20 minutes (Howard, 1978). This renders the timeframe for management actions aimed at destroying egg clutches very limited. After a period of two to four days the tadpoles hatch (Lougheed & Taylor, 2010; Stoutamire, 1932). In Canada, about 90 % of the eggs hatch (Govindarajulu et al., 2005).

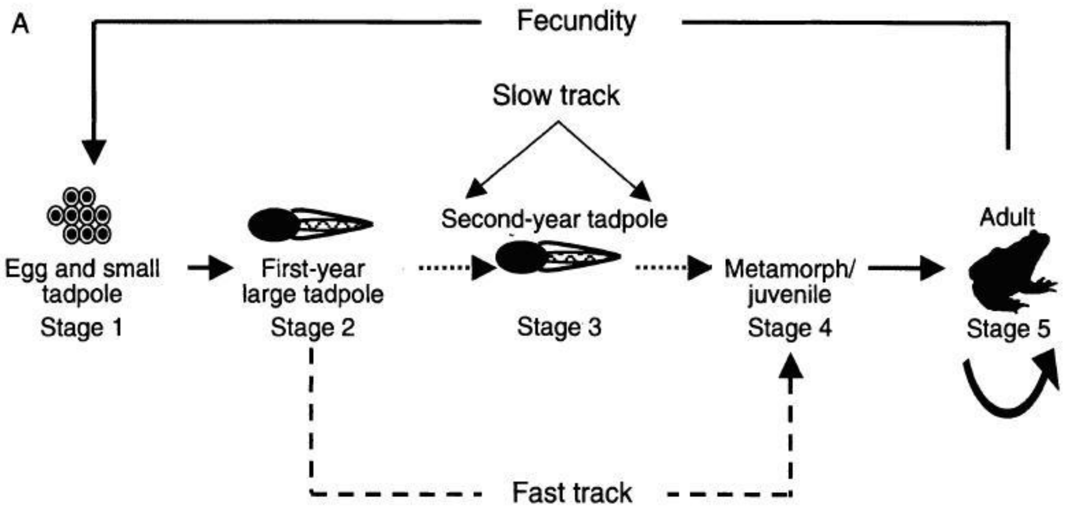


Figure 9: Bullfrog life cycle graph (for British Columbia) showing alternate pathways: slow track (dotted lines), where tadpoles attain metamorphosis after two years; fast track (dashed line), where tadpoles attain metamorphosis in one year. Figure from Govindarajulu et al. (2005).

Subadult males in most cases become sexually mature and start calling in their fourth year. Based on the intensity of chorus activity, mating in Flanders takes place late spring and early summer (Jooris 2005), corresponding with the breeding season of the species in Central Missouri where more than half of the females have deposited their eggs by the end of June (Willis et al, 1956). At the end of the mating ritual, the eggs are laid whilst the frogs are still in amplexus and are externally fertilized by the sperm of the male, which takes between 20 minutes and 2.5 hours to complete (Govindarajulu & Dodd, 2010). New generations of larvae appear in Belgium in the period July to September. These larvae metamorphose at the earliest in the summer of their second calendar year. Duration of the larval stage depends on various biotic and abiotic factors such as climate, water quality and water temperature, the density of conspecifics, food availability, predation pressure and timing of egg laying (Detaint & Coic, 2006; Wang & Li, 2009). The mean survival rates for larvae to metamorphosis in three populations of bullfrog were 11.8 %, 13.1 % and 17.6 % (Cecil & Just, 1979), which is high for anurans. Govindarajulu *et al*. (2004) have shown high density dependence in larval development. Increased predation pressure with subsequent decreasing density of larvae can cause an increase in the development rate of the remaining larvae (Abrams & Rowe, 1996; Govindarajulu et al., 2005). In this ‘fast developmental trajectory’ metamorphosis usually takes place around August of the first year, whereas in the slow developmental process this would occur only in July of the second or third year. The development of larva to metamorph in Flanders takes on average 2 years (Jooris, 2005), so at current there is no proof of the fast developmental track occurring in Belgium. Adults can become 7-10 years of age, possibly older (Stoutamire, 1932).

B/ Climatic requirements[2]

The bullfrog is considered a warm-adapted species (Spitzen-van der Sluijs & Zollinger, 2010a) and references therein). According to niche models, the probability of occurrence for bullfrog was highest for areas with a T_{min} (minimum temperature of the coldest month) ranging between –20 and +14°C (Ficetola et al., 2007b). Thus, bullfrogs are not present in areas with very cold winters (-20°C). Ficetola et al. (2007) also observed a positive relationship between bullfrog distribution and maximum temperature, with the species predicted to be present in areas having maximum temperatures > 20°C. Areas having high precipitations during both summer and winter, high maximum temperature, high human footprint (i.e. a measure of human influence combining data of population density, land transformation, human access, and the presence of infrastructures), and intermediate minimum annual temperature were those with the highest predicted suitability for bullfrogs. High summer temperatures (above 26°C) are preferred by the adults, which try to maintain a mean body temperature between 26-33°C but are capable of activity over a much broader thermal range (Lillywhite, 1970). Adults are often found sunning themselves on the shore or in shallow water. Below 15°C, adults are usually inactive and development is halted (Viparina & Just, 1975). In Belgium, bullfrogs generally become active between mid-April and early May. In October the animals go back into hibernation at the bottom of permanent water bodies, where they dig themselves in the mud or hide under stones (Jooris, 2005). As has been observed in Flanders, temporarily increased air temperatures can sometimes interrupt hibernation. The species has several adaptations to survive harsh winters, involving both behavioural adaptations (burrowing) and physiological responses (higher glucose levels) (Lougheed & Taylor, 2010; Rocha & Branco, 1998). In addition, adult bullfrogs can also resist substantial levels of hypoxia (Burggren & Mwalukoma, 1983).

In Belgium, the maritime temperate climate (Köppen-Geiger climate type *Cfb*) is characterized by fresh and humid summers and relatively mild and rainy winters. Precipitation is significant in all seasons like most of northwest Europe, with averages monthly rainfall of 74 mm for 2000-2006. The average temperature is lowest in January at 3°C and highest in July at 18°C. Since the beginning of the measurements, extreme temperatures of +40°C in the Campine area and -30°C in the valley of the Lesse have been recorded but such events are unusual (www.meteo.be). The absolute minimum annual average fluctuates between -10°C at the coast, -11°C to -14°C in low and middle Belgium, -15°C on the plateaus of upper Belgium and -19°C in the Ardennes valleys. The coastal and higher parts of the country are therefore considered less optimal for bullfrog establishment. Average precipitation is between 750-850 mm in lower and middle Belgium, 1,200-1,400 mm in higher Belgium. Under projected climate scenarios, by the end of the 21st century, the temperature is expected to increase with 1.7-4.9°C in winter (van Ypersele & Marbaix, 2004). Winter precipitation would also increase 6-23 % which is clearly in favour of bullfrog. The projected temperature increase in summer of 2.4-6.6°C is also in favour of the species. Summer precipitation projections range from status quo to a drop of 50 %. Extreme drought periods might occur more frequently. However, especially within the context of river valleys, we consider this as not problematic for bullfrog populations. Extreme drought events might lead to short periods of unsuccessful reproduction

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[2] Organism's capacity to establish a self-sustaining population under Atlantic temperate conditions (Cfb Köppen-Geiger climate type) should be considered, with a focus on its potential to survive cold periods during the wintertime (e.g. plant hardiness) and to reproduce taking into account the limited amount of heat available during the summertime.

because of lack of permanent water, but this effect is probably merely temporary and might be outweighed by higher developmental rates.

The long (at least > 12 years) persistence of introduced populations in Belgium shows that climatic conditions are not a limiting factor for successful establishment in the country. Breeding, dispersal, feeding and hibernation are all possible in Belgium.

C/ Habitat preferences[3]

Bullfrogs can occupy a broad range of wetland habitat types, including both artificial and natural wetlands, streams, lakes and temporary pools. They are usually relying on permanent water for breeding (Gahl et al., 2009; Stoutamire, 1932) and need at least two years of continuous water to develop to metamorphosis (Adams & Pearl, 2007). The water must be deep enough or provide a sufficiently thick silt layer to protect the frog against extreme weather conditions, without the pool drying out or freezing. High water temperature, e.g. in shallower parts of the habitat, and high nutrient load stimulate development (Lougheed & Taylor, 2010). Large, deep, turbid waters with dense aquatic and riparian vegetation are optimal for the species (Govindarajulu & Dodd, 2010). However, in Belgium, this seems only to be a minority of the populations, most of which are inhabiting small, shallow ponds. A lot of the water bodies used for breeding in Belgium are small, shallow fish ponds, with an average surface area of 1,500 m² and a maximum depth of 150 cm. The ponds are eutrophic, have high turbidity and contain no or very little submerged macrophytes, but often have a small belt of reed *Phragmites australis* and soft rush *Juncus effusus* along the shoreline. The fish community frequently contains large amounts of non-native species such as brown bullhead *Ameiurus nebulosus*, pumpkinseed *Lepomis gibbosus* and topmouth gudgeon *Pseudorasbora parva*, supplemented with native planktivorous species such as roach *Rutilus rutilus* and rudd *Scardinius erythrophthalmus* and benthivorous species like Prussian carp *Carassius gibelio* and common carp *Cyprinus carpio*. Piscivorous species like pike *Esox lucius* and perch *Perca fluviatilis* are mostly absent in these habitats. Native amphibians, notably common frog, common toad and green frog are sometimes present but in very low densities (pers. obs.). Although the length of the hydroperiod seems essential for successful reproduction, non-permanent ponds are an important habitat providing food and shelter for newly metamorphosed specimens (Gahl et al., 2009; Provenzano & Boone, 2009). In certain parts of the U.S. juvenile and adult bullfrogs are found in larger numbers in temporary ponds than in permanent ponds (pers. comm. G. Witmer).

D/ Food habits[4]

Larvae of the bullfrog mainly forage near the banks of the pool, in the warm upper layer of the water (Jooris, 2005). Larvae feed on periphyton, a mixture of algae (e.g. *Spirogyra* spp., *Euglena* spp., *Closterium* spp., *Volvox* spp. and *Scenedesmus* spp.) and detritus (Collins, 1979; Govindarajulu et al., 2004), and also small invertebrates (especially rotifers) (Ruibal & Laufer, 2012). Jooris (2005) showed that Belgian bullfrog larvae foraging in waters with a strong bloom of floating algae (phytoplankton)

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[3] Including host plant, soil conditions and other abiotic factors where appropriate.

[4] For animal species only.

achieved higher weights, were bigger and metamorphosed faster than larvae living in less nutrient-rich water.

Young and subadult individuals mainly feed on invertebrate prey, often macro-invertebrate tadpole predators such as dragonfly and beetle larvae. Adult bullfrogs are gape-limited generalist predators and eat anything they can manage from invertebrates and amphibians to fish, small rodents, reptiles and birds (Corse & Metter, 1980; Jancowski & Orchard, 2013). Da Silva *et al*. (2009) found, in an invasive south American population, that for adult bullfrogs, terrestrial prey were most abundant in the diet, with amphibious prey being most significant in volume. Bullfrogs are known to withstand highly evolved, mechanical and chemical prey defences such as stickleback spines, wasp stings and toxic repellents on newt or toad skin (Jancowski & Orchard, 2013; Silva et al., 2009). Prey on the bank is often approached from the water and jumped upon. Larger prey are drowned before they are swallowed. Bullfrog also prey, to a large extent, on their own eggs and larvae (see below). Martin (2009) showed that bullfrog from Ransart (Charleroi) fed on other bullfrog, gastropods (*Arion*, *Limax*, *Helix*), insects (especially Hemiptera, Gerridae), and to a lesser extent also crustaceans (crayfish) and arachnids. The animals used both aquatic and terrestrial prey as food (Martin, 2009).

E/ Control agents

A possible additional explanation for the success of American bullfrog as an invasive non-native species is the absence of natural enemies in the area in which they are introduced. In its native range - and probably the world - the species has relatively few natural enemies (Govindarajulu & Dodd, 2010; Lougheed & Taylor, 2010). According to Stoutamire (1932) bullfrogs are predated upon by fish, snakes, turtles, cats, skunks, hawks, herons as well as other birds of prey and predators. This is confirmed by several other literature sources (Adams & Pearl, 2007; Adams et al., 2003; Boone & Semlitsch, 2003). Furthermore, macro-invertebrates, especially the larvae of larger dragonflies (Odonata, Aeshnidae) and aquatic predatory beetle larvae (Coleoptera, Dytiscidae) are known to hunt on smaller larvae (< 20 mm) (Kiesecker & Blaustein, 1998). Both eggs and larvae of bullfrog are distasteful. Native predatory fish, such as pike and perch do eat bullfrog larvae (Louette, 2012), but prefer to eat larvae of native frog species when available (Jooris, 2005). Black bullhead (*Ameiurus melas*) also avoided bullfrog as prey items. Due to their relative unpalatability (Kats et al., 1988), bullfrogs can thrive perfectly in predator-rich waters. In Belgium, blue herons (*Ardea cinerea*) eat bullfrog larvae and possibly also adults, and also cormorants (*Phalacrocorax* carbo), which are known to prey on marsh frogs (pers. obs.) are potential predators of bullfrog tadpoles. Potentially, also other species of heron, such as black-crowned night-heron (*Nycticorax nycticorax*) and white stork (*Ciconia ciconia*), or mustelids like European polecat (*Mustela putorius*) that are known predators of native amphibians, might also predate on American bullfrog. In North America, leeches are the main predators of the egg clutches, especially *Macrobdella decora* (Howard, 1978). Adams and Pearl (2007) suggested the medicinal leech *Hirudo medicinalis* might play this role in Europe. However, this species being very rare in Belgium (pers. comm. J. Packet), this is unlikely to happen in the risk assessment area. Nevertheless, other leech species might play a significant role in egg predation. In the native range viral and bacterial infections, and a high water temperature are also a reported cause of mortality in embryos and larvae. Experiments with juvenile bullfrogs show that the animals experience heat stress from 38.2 °C (Cecil & Just, 1979). Because the larvae are distasteful to most vertebrate predators and adult bullfrogs show effective predator avoidance behaviour, it is estimated that predation is not a limiting factor for successful establishment of the species in Belgium, unless very low numbers of founders are involved.

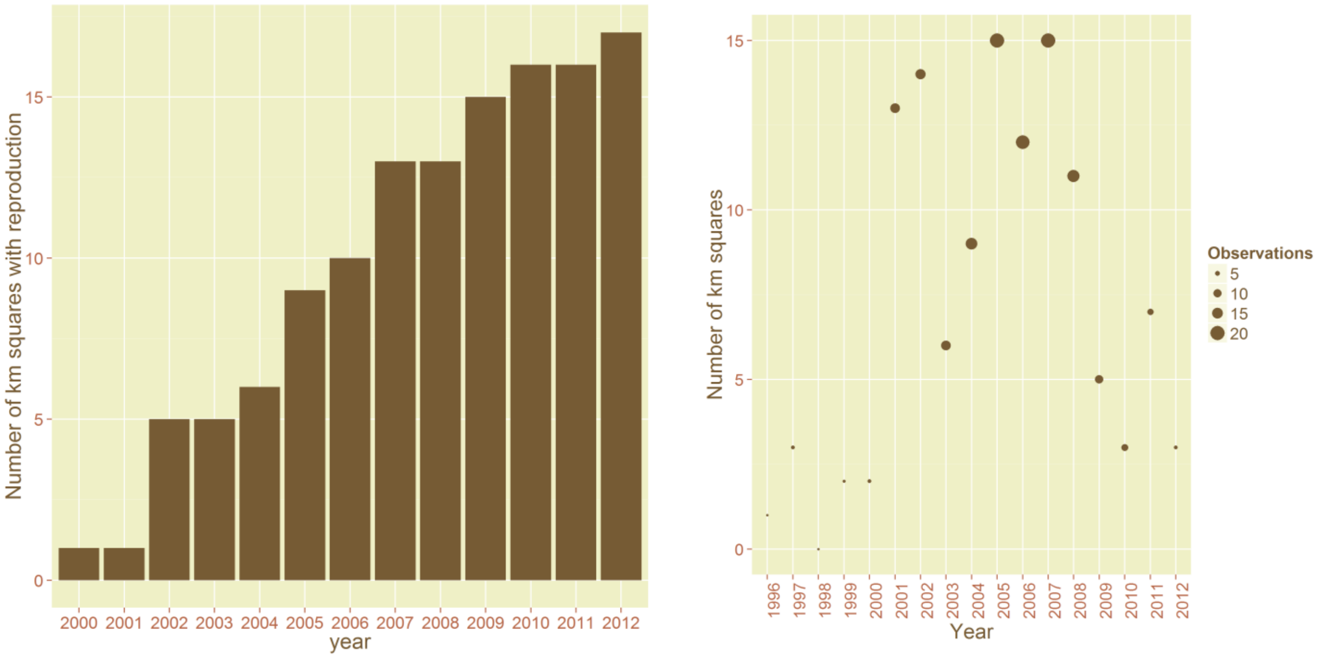
Cannibalism is a known cause of mortality in bullfrog (Bury & Whelan, 1984; Lougheed & Taylor, 2010). Adult bullfrogs are efficient predators of larvae, metamorphs and smaller adults and several sources indicate that cannibalism may limit population size (Govindarajulu & Dodd, 2010; Govindarajulu et al., 2004, 2005, 2006). However, other studies indicate that the importance of cannibalism varies across sites, season and year and suggest that, in the long-term, unmanaged populations might conceivably drive down native species numbers to a point where cannibalism becomes increasingly important to bullfrog population sustainability (Jancowski & Orchard, 2013). This is probably the case in Belgium, as confirmed by analyses of the diet of Walloon bullfrogs from Ransart. Taking into account the weight of different prey items, bullfrog contributed about 40 % to the diet of the analysed Walloon bullfrog adults (N = 10) (Martin, 2009). After the metamorphosis young bullfrogs disperse to suboptimal habitats such as small, sometimes strongly shaded ponds where little or no adult bullfrogs are present (Jooris, 2005). Chances are that this is a behavioural mechanism to escape cannibalism. Also, densities of native amphibians are extremely low in most of the bullfrog ponds, suggesting conspecifics might be relatively important prey.

F/ Establishment capacity in Belgium

American bullfrog is established in Belgium for more than a decade (see higher) and, as an established population, has been expanding its distribution area since 2000. Over the last decade, the area of occupancy (expressed as the number of occupied km^2 grid cells) increased to 17km^2 (Figure 8a). It has been observed in at least 70 km^2 grid cells in Belgium since 1996, but meanwhile disappeared from some of these through natural factors and, partly, eradication measures. It is also clear that the number of observations differs greatly between years and that during the last three years, the species is less frequently reported (Figure 10b).

The native and introduced range in North America includes ecoclimatic zones comparable to the risk assessment area. A niche model developed by Ficetola et al. (2007b) projects, under current climatic conditions, a high environmental suitability for invasion of American bullfrog in the central parts of Belgium, and medium suitability for the coastal, higher (Ardennes area) and southern parts of the country (Ficetola et al., 2007b).

It is to be noted that very few animals are needed to form new populations (Bai et al., 2012). Ficetola et al. (2008) showed that bullfrogs are capable of expansion and invasion even if their genetic diversity is very low. The number of founders for the French and Italian bullfrog populations was estimated at less than six founding females, showing that low critical propagule pressure is not a limiting factor for successful bullfrog establishment (Ficetola et al., 2008a). This ability to establish populations from only a handful of reproductive females is a challenge to the management of the species, emphasizing the need of rapid detection and removal.



a b

Figure 10: (a) Cumulative number of km^2 squares (Utm 1x1 grid cells) in Belgium with records of reproducing (= presence of tadpoles) American bullfrog since 2000 (data: Hyla, amphibian and reptile working group of Natuurpunt Studie and INBO-Invexo) (b) total number of Utm1x1km squares in Belgium with American bullfrog observations per year; the size of the dots is relative to the number of observations in that year.

G/ Endangered areas in Belgium

American bullfrog occurs in areas with high conservation value, such as Natura 2000 areas (Table 1).

Table 1: Designated Areas of the Natura 2000 network in Belgium with reported (reproducing) American bullfrog occurrences (data: Hyla, amphibian and reptile working group of Natuurpunt Studie, INBO-Invexo, Martin 2009).

Natura2000 code	**Name**	**Reproduction**
BE2100024	Vennen, heiden en moerassen rond Turnhout	+
BE2101437	De Maatjes, Wuustwezelheide en Groot Schietveld	-
BE2101538	Arendonk, Merksplas, Oud-Turnhout, Ravels en Turnhout	+
BE2221314	Hamonterheide, Hageven, Buitenheide, Stamprooierbroek en Mariahof	+
BE2422315	De Dijlevallei	+
BE2223316	De Demervallei	+
BE2100026	Valleigebied van de Kleine Nete met brongebieden, moerassen en heiden	-
BE2100040	Bovenloop van de Grote Nete met Zammelsbroek, Langdonken en Goor	+
BE2200032	Hageven met Dommelvallei, Beverbeekse heide, Warmbeek en Wateringen	+
BE2200033	Abeek met aangrenzende moerasgebieden	-
BE2400014	Demervallei	+
BE2100016	Klein en Groot Schietveld	-

BE2400011	Valleien van de Dijle, Laan en IJse met aangrenzende bos- en moerasgebieden	-
BE2300044	Bossen van het zuidoosten van de Zandleemstreek	-
BE31004	Vallée de la Dyle en aval d'Archennes	+

The species has also invaded nature reserves (Table 2).

Table 2: Nature reserves in Belgium with reported American bullfrog occurrences (data: Hyla, amphibian and reptile working group of Natuurpunt Studie, INBO-Invexo, Martin 2009).

Nature reserve	**Population status**
Groot Schietveld	Solitary Introduction
Goorken/Lokkerse Dammen/Rode Del	Established
Zammelsbroek	Established
Hageven	Presumably established
Griesbroek	Established
Kwarekken	Established
Roost-Craeywinckel	Established
Vijvers van Florival	Solitary Introduction

To evaluate whether bullfrogs occupy areas of conservation concern, we followed a third approach using the Biological Valuation Map (BVM, version 2.0) which is available for Flanders only (Wils et al., 2004). The BVM is a uniform survey of the land cover and vegetation (ecotope level) which is translated into a biological valuation, largely based on the presence of indicator plant species and vegetations. The biological value of legend units is fixed and determined by a number of ecological criteria: rarity of the biotope, presence or absence of certain (key) species, biodiversity of the biotope, vulnerability and replaceability of the biotope. We calculated the surface area of *very valuable*, *valuable* and *valuable with very valuable elements* land with reported occurrences of bullfrog. From this intersect it becomes clear that bullfrog is present in (the vicinity of) parcels with valuable ecotopes (Figure 11). Among infected *very valuable* ecotopes are:

- eutrophic ponds with diverse plant communities (BVM code *ae*, 40 records),
- moist willow thickets on nutrient rich soils (code *sf*, 9 records),
- reedlands (code *mr*, 5 records),
- mesotrophic ponds (*aome**, 4 records).

Parcels with bullfrog that scored *valuable* mostly contained species rich meadow complex with ditches and micro-relief (*hpr**). From these habitats, there is clearly good potential for bullfrogs in Belgium to encounter native amphibian species. As to the impact on species of conservation concern, such as red list and/or Habitats Directive species (e.g. great crested newt *Triturus cristatus*, fire salamander *Salamandra salamandra*, common spadefoot *Pelobates fuscus*, European tree frog *Hyla arborea*, common midwife toad *Alytes obstetricans* or natterjack toad *Epidalea calamita*), further research is needed to evaluate the risk posed by the presence of bullfrog on the conservation status of their populations. Currently, the species has the greatest niche overlap with more common species like green frog *Pelophylax* kl. *esculentus* and common toad *Bufo bufo* (permanent, eutrophic ponds).

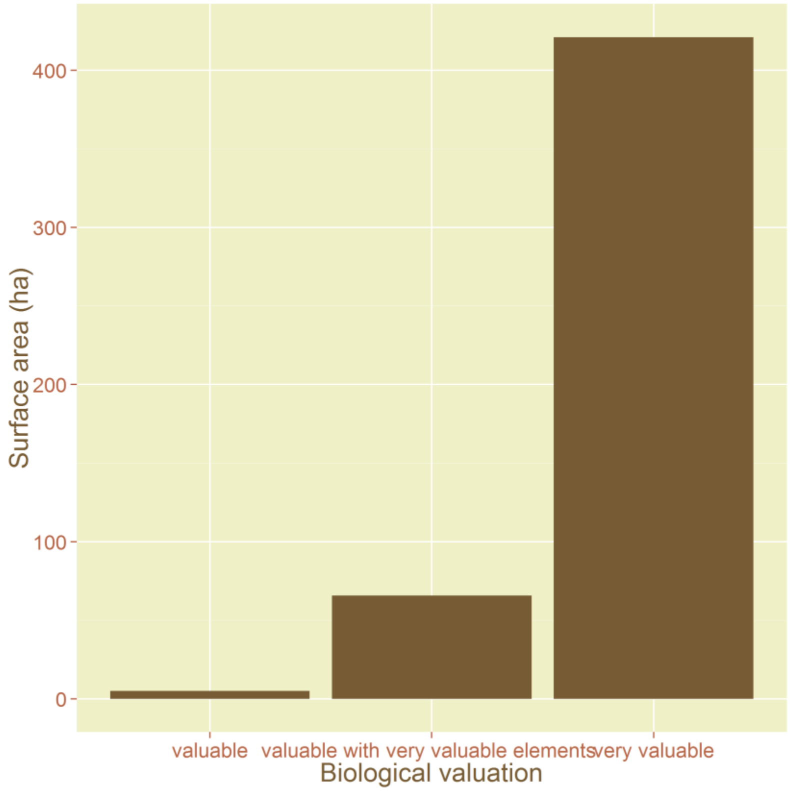


Figure 11: Surface area of different valuation categories according to the biological valuation map (BVM) on parcels where bullfrogs occur in Flanders (northern Belgium).

Establishment capacity in the Belgian geographic districts:

Districts in Belgium	**Environmental conditions for species establishment**[5]
Maritime	Inadequate
Flandrian	Suboptimal
Brabant	Optimal
Kempen	Optimal
Meuse	Optimal
Ardenne	Inadequate
Lorraine	Suboptimal

ESTABLISHMENT CAPACITY AND ENDANGERED AREAS IN BELGIUM

American bullfrog has flexible life-history strategies in favour of successful establishment from only a very limited number of founders. It is a generalist species that can occupy a broad range of wetland habitat types and profits from human interference in the landscape. The species has a high reproductive capacity and few natural enemies in Belgium. Belgian climatic variables are well within it physiological requirements and climatic conditions are not limiting for successful establishment. Meanwhile, it is well established in Belgium for more than a decade and has invaded areas of conservation concern.

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[5] For each district, choose one of the following options : optimal, suboptimal or inadequate.

2.1.5 Dispersion capacity

Specify what is the rate of dispersal once the species is released or disperses into a new area. When available, data on mean expansion rate in introduced territories can be specified. For natural dispersion, provide information about frequency and range of long-distance movements (i.e. species capacity to colonise remote areas) and potential barriers for spread, both in native and in introduced areas, and specify if the species is considered as rather sedentary or mobile. For human-assisted dispersion, specify the likelihood and the frequency of intentional and accidental movements, considering especially the transport to areas from which the species may easily colonise habitats with a high conservation value.

A/ Natural spread

Post-metamorphic stages are capable of dispersing long distances and are typically colonizing new sites (> 1,200 m) (Willis et al., 1956). Data inferred from the reproducing populations (largely Grote Nete Valley in Flanders) show that the species can easily spread within the context of a river system with numerous suitable artificial pond habitats. The average increase in the area of occupancy (expressed as the number of occupied 1x1km^2 grid cells) was 1.5 grid cells per year for the period 2000-2012 (Figure 10). Maximum distances recorded with capture mark recapture studies of adult individuals are 1,600, 914 and 966 meter (Smith & Green, 2005). Radio telemetry was performed on 9 individuals (4 male, 5 female) in Flanders, showing a degree of homing behaviour in adult bullfrogs with enhanced dispersal activity during the breeding season, to a maximum of 1,500 m (Descamps & De Vocht, 2012). Natural dispersal is rapid in comparison with other amphibians (individual movements of > 3 km) (Anon., 2011). Global spread has been especially rapid due to human assistance (e.g. Baker, 1999). Evidence from other non-range states indicates spread may be rapid. In south western France, following initial introduction in the 1960s, the species is now distributed over more than 2000 km^2 (Ficetola et al., 2007a). Introductions in Flanders show that dispersal to nearby sites occurs frequently, aided by river valley corridors (Jooris, 2005), small ditches or swampy forest (Descamps & De Vocht, 2012). However, locally, certain infrastructures might represent barriers to bullfrog dispersal, such as broad canals (Jooris, 2005) or high levels of urbanisation (Martin, 2009).

B/ Human assistance

Successful establishment following translocation by humans is known in continental Europe and other non-native range areas. Evidence in other non-native ranges indicates that translocation by humans often complements natural spread. The species is often seen as charismatic and may be subject to collection and release by interested members of the public, or may be spread unintentionally (Ficetola et al. 2007a,b). Also, in Belgium, there is evidence that at least some populations are the result of human introductions, at places where colonisation would be unlikely (e.g. Ransart, solitary indidividuals in western and eastern Flanders), thereby nullifying any potential dispersal limitations that might occur naturally.

DISPERSAL CAPACITY
Natural dispersal in American bullfrog can be considerable (>1km/year). Translocation by humans often complements natural spread.

2.2 EFFECTS OF ESTABLISHMENT

Consider the potential of the non-native organism to cause direct and indirect environmental, economic and social damages as a result of establishment. Information should be obtained from areas where the pest occurs naturally or has been introduced, preferably within Belgium and neighbouring areas or in other areas with similar eco-climatic conditions. Compare this information with the situation in the risk analysis area. Invasion histories of comparable organisms can be considered if useful. The magnitude of those effects should be compared with those caused by their closest native relatives.

2.2.1 Environmental impacts

Specify if competition, predation (or herbivory), pathogen pollution and genetic effects are likely to cause a strong, widespread and persistent decline of the populations of native species and if those mechanisms are likely to affect common or threatened species. Document also the effects (intensity, frequency and persistency) the non-native species may have on habitat peculiarities and ecosystem functions, including physical modification of the habitat, change to nutrient cycling and availability, alteration of natural successions and disruption of trophic and mutualistic interactions. Specify the kind of ecosystems that are specifically at risk.

A/ Competition [HIGH]

Their numerical advantage, combined with their larger size and correspondingly higher metabolic needs, makes bullfrogs formidable competitors (Kiesecker et al., 2001; Wang et al., 2007). There is strong field observational and experimental evidence that bullfrogs compete with native ranid frogs for resources (Bury & Whelan, 1984; Fisher & Shaffer, 2002; Kiesecker & Blaustein, 1998; Kiesecker et al., 2001; Kupferberg, 1997; Lawler et al., 1999; Moyle, 1973). For a synthesis of literature on this subject see also Bomford *et al*. (2005). Competitive effects are especially well demonstrated in the larval stage, for which competition appears to be mediated primarily by algal resources. The larvae of bullfrog are highly competitive with native amphibian larvae, inhibiting their growth and development, which can have manifold effects on their fitness (e.g. Werner, 1994). Kupferberg (1997) experimentally showed strong negative effects of bullfrog larvae on survival and size at metamorphosis in two native species. Survival of native yellow-legged frog *Rana boylii* tadpoles was reduced by 48 % and the weight of the metamorphs by 24 % in the presence of bullfrog larvae, whereas the impact on Pacific treefrogs *Hyla regilla* was smaller, leading to a 16 % reduction in metamorph mass, and no significant effect on survival (Kupferberg 1997). *Rana boylii* was also found to be almost an order of magnitude less abundant in reaches where bullfrogs were well established. Responses to bullfrogs in field settings were similar to the results from small-scale experiments, with competition from large overwintering bullfrog larvae significantly decreasing survival and growth of native tadpoles (Boone et al., 2004). Lawler et al. (2001) found that, in the presence of bullfrog tadpoles, survivorship of tadpoles of the California red-legged frog *R. draytonii* was reduced, suggesting a strong impact of bullfrog on the decline of this rare species (Lawler et al., 1999). Bullfrogs nearly eliminated red-legged frog recruitment in this experiment. As for native amphibian populations in Belgium and Northwest Europe, green frog *Pelophylax* kl. *esculentus* and common toad *Bufo bufo* can be expected to be adversely affected by competition and predation because of their overlap in habitat (permanent, eutrophic ponds) (Louette & Bauwens, 2013). Other native amphibian species are less likely to come into direct contact with American bullfrogs because they usually depend on a different kind of reproductive habitat (temporary and fishless water). These species, however, can still experience impact by the transmission of viruses and fungi (see further).

Besides affecting growth and development, tadpoles of native species can also alter their microhabitat use in the presence of bullfrog adults and tadpoles, which alters their susceptibility to predation and development (Kiesecker & Blaustein, 1998). Clumped (as opposed to uniformly scattered) resources, that are often found in artificial or human-influenced aquatic habitats, can intensify interspecific competition (Kiesecker et al., 2001). The possible impacts of adult terrestrial bullfrogs as competitors are considerable but difficult to quantify (Bomford et al., 2005). Some authors found a high degree of diet overlap between juvenile terrestrial bullfrogs and adult native frogs (Morey & Guinn, 1992), but the extent to which this resource competition limits native frog populations is unknown. Yiming et al. (2011) found that invaded sites had lower native frog density and native species richness than uninvaded sites. Their models for Zhoushan archipelago (East China Sea) showed that post-metamorphosis bullfrog density was the most important factor correlating with this. As in this study area larvae were only present in a very limited number of ponds, the authors suggest a combined effect of predation on native species and competition for food resources (Yiming et al., 2011).

A well-known European example of displacement of native species by American bullfrog is the case of the Cretan frog (*Pelophylax cretensis*), an endemic on Crete. Bullfrog were introduced in Agia Lake in 2000, resulting in local extinction of this frog species. Again, this red list species is primarily threatened by habitat loss and the destruction of water resources, but the presence of bullfrog poses an additional pressure (Beerli et al., 2009).

Concern has also been raised about disturbance of the reproduction of native species by American bullfrog through breeding interference. In the west of the US range, where bullfrog is considered invasive, interspecific amplexus with native *Rana* species occurs relatively frequently (D'Amore et al., 2009; Pearl et al., 2005). Males thereby typically mistake the bigger juvenile bullfrogs with females of the same species, which causes time and energy loss as well as the removal of males from the pool of reproductive animals. In populations with skewed sex ratios and a short, intense period of reproduction - which is typically accompanied by prolonged amplexus - this could impact on the success of individuals and eventually populations. This might influence already declining species like *Rana pretiosa*, an explosive reproducer for which in some populations a shortage of males occurs (Pearl et al., 2005). In Belgium, interspecific amplexus of common toad with juvenile American bullfrog has been documented (Adriaens et al., 2011), and there is field observational evidence for interspecific amplexus with green frog (pers. comm. J. Van Der Voort and R. Jooris). However, the risk of intensive breeding interference leading to effects at population level of native species in Belgium can be considered low since densities of both adult bullfrogs (see higher) and native species at infected sites are relatively low, thereby reducing the probability of interspecific mating encounters. Moreover, *B. bufo* populations exhibit, like most frog and toad populations, a surplus of males (e.g. Cooke & Oldham, 1995).

Another way of breeding interference is by invasion of the acoustic niche of native species. In Brazil, native tree frog (*Hypsiboas albomarginatus*) males shifted to higher frequencies when exposed to recorded invasive American bullfrog calls. The dominant and centre frequencies of tree frog calls were affected, thereby potentially negatively affecting their reproductive success (Both & Grant 2012). The authors argue that the effect of bullfrog vocalizations on acoustic communities can be expected to be severe due to their broad frequency band, which masks the calls of multiple species simultaneously. Whether such acoustic interference occurs with native species in Belgium is unknown. If such occurs, it would at current only affect species of least concern, such as green frogs *Pelophylax* spp. or common toad *Bufo bufo*, with which is frequently co-occurs (Louette & Bauwens 2013). The impact of such under the scenario that American bullfrog were to realise all of its potential niche in Belgium, is impossible to evaluate.

B/ Predation [HIGH]

Due to their relative size and voracious behaviour, both on conspecifics and other species, American bullfrogs are top predators in their introduced range (Kats & Ferrer, 2003). Adult bullfrogs are gape-limited, generalist carnivores, eating any animal smaller than themselves, mainly crustaceans and insects, but also rodents, bats, frogs, birds, fish and reptiles (Bury & Whelan, 1984; Rosen & Schwalbe, 1995). Tadpoles are strongly herbivorous, mainly eating detritus and algae (periphyton), although in the laboratory tadpoles also ate the eggs and tadpoles of the native frog *Rana blairi* (Bury & Whelan, 1984). Predation by bullfrog can result in reduction, elimination or displacement of native species, as has been shown by numerous authors (Adams & Pearl, 2007; Bradford, 1991; Bradford, 2002; Bradford et al., 1994; Bradford et al., 2004; Bradford et al., 2005; D'Amore, 2012; Hayes & Jennings, 1986; Kiesecker et al., 2001; Pearl et al., 2004; Rosen & Schwalbe, 1995). Introduced bullfrogs have been blamed for amphibian declines in much of western North America (Bury & Whelan, 1984; Hayes & Jennings, 1986; Lever, 2003; Vial & Saylor, 1993). Its predatory habits have been implicated in the decline of several native ranid frog species and one snake species (Bury & Whelan, 1984; Corn, 1994; Kupferberg, 1997; Moyle, 1973; Schwalbe & Rosen, 1988). Several removal experiments have also shown spectacular recovery of native species after bullfrogs were removed or eradicated from a site, which could be attributed to both behavioural changes and increased population sizes of native species (D'Amore et al., 2009; Hecnar & M'Closkey, 1997; Rosen & Schwalbe, 1995).

Besides direct predation, there is evidence for complex biotic interactions with native amphibians caused by the presence of bullfrogs. When bullfrogs are present the tadpoles of native frogs can alter their habitat use, thereby becoming more vulnerable to the predation by fish (Blaustein & Kiesecker, 2002). Studies have also shown that growth rates of amphibian larvae are reduced and larvae metamorphose at smaller sizes when alien predators such as bullfrog are present (Boone et al., 2004; Kats & Ferrer, 2003; Kupferberg, 1997; Li et al., 2010). Also, as an indirect effect of predation, native species often have to migrate to other, suboptimal, habitats to avoid being eaten. Boone et al. (2007) showed migrations from shallow, warm, and safe riparian water to deeper and colder water further from the shore. This way, native species experience reduced food supply, increased exposure to predators and lower water temperatures which cause lower developmental rates. The unpalatable nature of bullfrog larvae (Kats et al., 1988; Kruse & Francis, 1977), allows them to develop without much resistance, which again increases the pressure on native species (Kiesecker & Blaustein, 1998).

Although bullfrogs are undoubtedly significant aquatic predators, it is unclear whether the general lack of other biota at some of the Belgian bullfrog sites is solely attributable to their presence. Most probably, predation is acting complementary to habitat change here. Also, the presence of non-native fish can act as a facilitating factor in this respect, leading to a cumulative predation effect impacting on survival and development of native species (see further) (Adams, 2000; Adams et al., 2003; Boone et al., 2007).

C/ Genetic effects and hybridization [LOW]

No published information was found on potential genetic effects of American bullfrogs on other biota. There is no evidence that bullfrogs could successfully breed with native species (Anon., 2011).

D/ Pathogen pollution [HIGH]

American bullfrogs are known vectors of *Batrachochytrium dendrobatidis* (*Bd*) (Cunningham et al., 2005; Daszak et al., 2004; Fisher & Garner, 2007; Garner et al., 2006). This chytrid fungus is the cause of the infectious amphibian disease chytridiomycosis, that has caused widespread declines in amphibian populations globally and caused the extinction of several Neotropical and Australian species (Berger et al., 1998; Daszak et al., 1999). There is strong evidence that the amphibian trade is contributing to the spread of this pathogen (Fisher & Garner, 2007; Picco & Collins, 2008). Although the relative importance of *Bd* as a causative agent of amphibian declines is currently under some debate (cf. Heard et al., 2011; McCallum, 2005), the 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 & Martínez-Solano, 2006) in Spain. The fungus has been shown to be widely distributed in Europe (Olson et al., 2013) but also Belgium and the Netherlands (Figure 12). A large number of skin swabs have shown 4 % of all tested amphibians, including several red list species, infected with *Bd* (Spitzen-Van der Sluijs et al., 2010), urging the need for a more thorough investigation into the sensitivity for and the impact of *Bd* on the native amphibian fauna, preventive screening for amphibians in pet trade and reintroduction projects, as well as pathogen monitoring of sensitive species (Fisher & Garner, 2007; Fisher et al., 2012; Pasmans et al., 2006; Schloegel et al., 2012; Spitzen-Van der Sluijs et al., 2010). In 2010, evidence became clear of a first case of mortality in a wild population of common midwife toads in Belgium due to chytridiomycosis (Pasmans & Martel, 2011; Pasmans et al., 2010).

Some adult bullfrogs and tadpoles that were caught during the recent eradication campaigns in Flanders and The Netherlands were checked for pathogens. By means of quantitative (q)PCR, the amount of *Bd*-DNA in the mouthparts of 88 bullfrog larvae and skin swabs of 164 (sub)adult bullfrogs was determined. *Bd* was detected in 18 of the 88 larvae (20.5 %) and in 104 of the 164 frogs (63.4 %). This confirms the role bullfrogs play in the maintenance and spread of *Bd* infection in communities of amphibians in Belgium. However, it remains unknown whether the *Bd* strains in the Belgian bullfrogs belong to the hyper-virulent panzootic line that poses a serious worldwide threat for several amphibian populations (Pasmans & Martel, 2012).

Figure 12: Sampled sites for *Bd*-infected native amphibians and American bullfrog in The Netherlands and Flanders. Red dots represent sites that tested positive for the presence of *B. dendrobatidis* (Spitzen-Van der Sluijs et al., 2010).

Besides chytrid fungus, American bullfrogs are carriers of at least one fatal *Ranavirus* (Daszak et al., 1999; Mazzoni et al., 2009; Schloegel et al., 2009). The link between the decline of amphibian populations and ranavirosis is less clear than in chytridiomycosis. Yet, this viral disease is considered a potential threat to amphibian populations, as it can cause a high mortality in all life stages by exhibiting viral lesions to liver, kidneys and spleen (Daszak et al., 1999; Densmore & Green, 2007). Older animals can carry the virus but will in most cases not die and the disease is transmitted from one species to another, even outside the Ranidae family (Mazzoni et al., 2009). However, a recent study has linked Ranavirus with mass die-off of over 1000 wild water frogs *Pelophylax* spp. and at least 10 common newts *Lissotriton vulgaris* in a pond in The Netherlands (Kik et al., 2011). The FV3-Virus (Frog Virus 3) is part of the Iridoviridae family and is highly pathogenic and virulent to the larvae of American bullfrog, pig frog *L. grylio*, *Rana tigrina*, *R. sylvatica* and *R. pipiens* but also to the larvae of species that occur in Belgium like common frog, green frog, common toad, common midwife toad and alpine newt *Ichthyosaura alpestris* (Mazzoni et al., 2009; Miller et al., 2011). Although timing and severity of an outbreak are influenced by environmental factors, genetic diversity and stress (Flores-Nava, 2005; Miller et al., 2011), for some of these species, in Europe, mass mortalities have been assigned to FV3. The bullfrog population in Hoogstraten also showed infection with *Ranavirus*. Of the 400 tested larvae 0.75 % appeared to be a carrier of this virus, without displaying clinical symptoms (Sharifian-Fard et al., 2011). This shows that the bullfrog in Flanders, as in the United States (Adams & Pearl, 2007), can act as a reservoir for ranaviruses. Unlike the Flemish samples, none of the larvae from The Netherlands tested positive for *Ranavirus* infection (Sharifian-Fard et al., 2011).

Moreover, Martel et al. (2012) identified a novel genus and species of *Chlamydiales* (Candidatus *Amphibiichlamydia ranarum*), with a prevalence of 71% (N = 200), in bullfrog tadpoles from the

Baarlo population in the Netherlands. This chlamydia was not present on tested livers of tadpoles from the Flemish eradication programme. Although none of the examined tadpoles showed signs of clinical disease, the authors urge for evaluation of its pathogenic potential for native amphibian species (Martel et al., 2012).

The impact of *Bd*, *Ranavirus* and *Amphibiichlamydia* on native amphibians in Belgium is not yet fully unravelled. Nevertheless the potential for pathogen pollution in the risk assessment area is clearly high as demonstrated above. It should be noted that American bullfrog is not the only carrier of *Bd* which can also be transmitted by other species like Canada goose (*Branta canadensis*) (Garmyn et al., 2012) or species of crayfish *Procambarus* spp. (McMahon et al., 2013), with which bullfrogs co-occur in Belgium (unpubl. data).

E/ Effects on ecosystem structure and functioning [LIKELY]

Habitat alteration and changes in nutrient cycling

Seale (1980) reported that introduced American bullfrog tadpoles significantly reduced the rate of primary production of phytoplankton in a pond. Moreover, the presence of tadpoles altered species composition and shifted the nitrogen state from particulate to dissolved (Seale, 1980). Kupferberg (1997) showed that tadpoles, in a northern California river system, significantly affected benthic algae, although effects varied across sites.

Interactions with other invasive non-native species

Adams et al. (2003) showed invasion of bullfrogs to be facilitated by the presence of co-evolved non-native fish, which increased tadpole survival by reducing predatory macro-invertebrate densities. Native dragonfly nymphs in Oregon, United States caused zero survival of bullfrog tadpoles in a replicated field experiment, unless non-native bluegill *Lepomis macrochirus* were present to reduce dragonfly density. This pattern was also evident in pond surveys where the best predictors of bullfrog abundance were the presence of non-native fish and bathymetry (Adams et al., 2003). Such positive interactions among non-native species have the potential to disrupt ecosystems via indirect mechanisms (facilitation, predator release) by amplifying invasions. In Belgium, bullfrogs live mostly in nutrient rich, turbid ponds with low vegetation structure (no submerged macrophytes) and high abundance of non-native fish species like pumpkinseed sunfish and topmouth gudgeon. These fish species are well known predators of macro-invertebrate and zooplankton communities in aquatic ecosystems (e.g. Anseeuw, 2011). Their presence can thereby both reduce the predation pressure by macro-invertebrates (e.g. Dytiscidae, Aeshnidae) on tadpoles (predation release) and increase the food availability for bullfrog larvae through an increase in phytoplankton (facilitation) (Adams & Pearl, 2007; Adams et al., 2003). Management efforts combining the reduction of both bullfrog numbers and exotic fish such as pumpkinseed, will therefore enhance the success rate (Spitzen-Van der Sluijs et al., 2010). It has also been suggested that freshwater habitats with diverse predator communities may be more resistant to invasion by American bullfrog. Tadpoles can suffer higher predation when inexperienced with a certain (native) predator and can therefore be unable to assess predation risk and invoke anti-predator responses (Garcia et al., 2012).

There is some debate in Belgium as to whether American bullfrog are really the drivers of ecological change in freshwater ecosystems or rather passengers that can profit from changed habitat conditions (e.g. habitat modification, introduced fish, altered hydrology). More notably, there is evidence that introduced fish can have larger impacts on native amphibian communities than bullfrogs in areas where fish and bullfrogs co-occur (Adams, 1999). Also, non-native fish could bring freshwater ecosystems in a turbid state by predating on zooplankton. Despite this, the cumulative direct and indirect effects of bullfrog presence act synergistic and add another stressor to native amphibian populations (D'Amore, 2012).

ENVIRONMENTAL IMPACTS

There is substantial evidence that American bullfrog may negatively affect native amphibians through competition for resources and through direct and indirect predation effects. The effects will be enhanced when species are under pressure from other factors, such as habitat loss, fragmentation, habitat degradation or the presence of invasive fish. Moreover, the bullfrog is a vector of a number of important amphibian diseases that have been implicated in amphibian declines in Europe.

2.2.2 Other impacts

A/ Economic impacts

Describe the expected or observed (in)direct costs of the introduced species on sectorial activities (e.g. damages to crops, forests, livestock, aquaculture, tourism or infrastructures).

Although no reports of harm to aquaculture facilities in Belgium caused by exotic amphibians were found, the potential for such harm does exist (Bomford et al., 2005). American bullfrog caused considerable damage to a fish hatchery in Missouri used for raising goldfish *Carassius auratus* for aquarium trade and golden shiners *Notemigonus chrysoleucus* as fish bait. Both direct predation by adult bullfrogs on fish and eating of commercial food provided for the fish by tadpoles caused an economic loss calculated at $US 42.000 per year (Corse & Metter, 1980). In theory, bullfrogs could inflict similar damage to aquaculture facilities in Belgium.

The indirect costs for the control and management of the species can be considerable (see 2.1.2 Present status in neighbouring countries, with figures of eradication efforts in GB and Germany). The exact price for management of bullfrog in its entire invasive range is impossible to calculate. Moreover, numbers are hardly comparable between countries as different costs are involved for different management measures (e.g. shooting adults versus drainage of ponds), the application of which is often insufficiently documented. Moreover, it is often impossible to separate preparatory costs and expenses for monitoring, inventory, actions in the field, communication and awareness raising, research, overhead, purchase of land and follow-up. However, in Britain £ 100,000 (€ 116,990) was spent on eradication of the species over four years (1999-2002) (Foster & Banks, 2008). In Germany, the cost of eradicating five isolated populations in the Upper Rhine Valley was estimated at € 270.000, averaging € 53,000 per pond annually (Reinhardt et al., 2003). The same authors assessed that this figure would rise to 4.4 billion if control measures were needed throughout the whole country. Canada spent € 136,912 spread over four years (2007-2010) to fight bullfrog (Orchard, 2010). In the Netherlands, a two year project (2011-2012) to remove two breeding populations has cost about € 50.000 (pers. comm. J. Vos). In Flanders, € 390,500 was spent on bullfrog activities in the context of an EU funded Interreg project (www.invexo.eu), including costs for research, inventory, early warning activities and actions in the field using both drainage, hand captures and double fyke netting at six ponds (pers. comm. E. van den Broeke) (Devisscher et al., 2012). The estimated cost for restoring the six ponds and eliminating the bullfrog population was € 85,000.

B/ Social impacts

Describe the expected or observed effects of the introduced species on human health and well-being, recreation activities and aesthetic values.

Since American bullfrog occur in fish and garden ponds, there is a potential for both direct and indirect contact with people. Bullfrogs could thus represent a source of microbial pathogens to humans. Flemish and Dutch animals that were caught within an eradication campaign (2009-2012) were tested on a number of zoonotic pathogens that can cause infectious diseases transmissible from animals to humans. Animals from Hoogstraten, Arendonk (Belgium) and Baarlo (The Netherlands) were tested for *Coxiella burnetii*, *Neospora caninum*, *Leptospira* sp., *Toxoplasma gondii*, *Mycoplasma* sp., *Campylobacter* sp., *Salmonella* sp. and extended-spectrum beta-lactamase producing *Escherichia coli*. None of the 164 animals examined from six different sites showed traces of these diseases, suggesting a limited role of bullfrogs as carrier of zoonotic pathogens (Martel et al. 2013).

Based on interviews with private pond owners and fishermen that cooperated within an EU-Interreg funded eradication project (www.invexo.eu), bullfrogs are sometimes regarded as a nuisance species in eutrophic ponds used for recreational fishing. This is primarily due to the high densities of bullfrog larvae that can be attained in such habitats, the presence of which is perceived as a negative influence on the quality of the pond as a habitat for fish and/or aesthetic value of the pond. Some newspaper articles in Flanders and The Netherlands bear witness to nuisance caused by male vocalisations in the breeding period.

2.2.3 Summary of the environmental risk assessment

Fill in the following table with the conclusions provided at the end of the different sections of the risk assessment.

PROBABILITY OF ESTABLISHMENT AND SPREAD (EXPOSURE)	
Entry in Belgium	The pathways of introduction in Belgium remain largely undocumented. The risk of escaping American bullfrogs from captivity is currently considered low as compared to other introduction pathways such as deliberate introductions for ornamental purposes and accidental introduction through contaminated fish lots. Natural colonisation from neighbouring countries is considered unlikely.
Establishment capacity	American bullfrog has flexible life-history strategies in favour of successful establishment from only a very limited number of founders. It is a generalist species that can occupy a broad range wetland habitat types and profits from human interference in the landscape. The species has a high reproductive capacity and few natural enemies in Belgium. Belgian climatic variables are well within it physiological requirements and climatic conditions are not limiting successful establishment. Meanwhile, it is well established in Belgium for more than a decade and has invaded areas of conservation concern.
Dispersion capacity	Natural dispersal in American bullfrog can be considerable (> 1km/year). Translocation by humans often complements natural spread.
EFFECT OF ESTABLISHMENT	
Environmental impacts	There is substantial evidence that American bullfrog may negatively affect native amphibians through competition for resources and through direct and indirect predation effects. The effects will be enhanced when species are under pressure from other factors, such as habitat loss, fragmentation, habitat degradation or the presence of invasive fish. Moreover, the bullfrog is a vector of a number of important amphibian diseases that have been implicated in amphibian declines in Europe and throughout the world.

STAGE 3: RISK MANAGEMENT

The decision to be made in the risk management process will be based on the information collected during the two preceding stages, e.g. reason for initiating the process, estimation of probability of introduction and evaluation of potential consequences of introduction in Belgium. If the risk is found to be unacceptable, then possible preventive and control actions should be identified to mitigate the impact of the non-native organism and reduce the risk below an acceptable level. Specify the efficiency of potential measures for risk reduction.

3.1 RELATIVE IMPORTANCE OF PATHWAYS FOR INVASIVE SPECIES ENTRY IN BELGIUM

The relative importance of intentional and unintentional introduction pathways mediated by human activities should be compared with the natural spread of the organism. Make use e.g. of information used to answer to question 2.1.3.

Two main pathways for colonization of new habitats by American bullfrog can be identified. These include both natural dispersal, with an average dispersal rate of around 1 km per year, as well as deliberate introduction by humans (covering several tens of kilometers). Natural spread is ongoing and up till now not stopped by the eradication actions. Natural dispersal seems only to be halted or slowed down when dispersal barriers are present (e.g. canals, urbanised areas, lack of reproduction sites). Deliberate releases of animals still tends to be important in explaining new introductions outside the big populations that are already present for a longer period in Belgium, cf. the relatively high number of solitary individuals reported outside the river valley populations. Also, we cannot exclude that, even within the known core populations, human-mediated (deliberate/accidental) translocation of animals is contributing to the spread of the species. Bullfrog larvae can be introduced as stowaways in fish lots. Especially fish used for stocking public waters and rivers can be an important pathway, as lots with ornamental fish (goldfish, koi) are normally sorted before going to retailers (what happens with bullfrog larvae, if present, is unknown). Most of the fish used for stocking in Flanders originates from eastern European regions (pers. comm. H. Verreycken). American bullfrog is thought to be absent there, but contamination of these lots potentially happens at stop-overs of the fish in Flemish ponds. Although some discouragement for deliberate introductions can be invoked through the media (e.g. press releases issued during the Invexo Interreg project, for an overview see Devisscher *et al*. 2012), a legal basis for prevention of deliberate release can complement such preventive actions and form the basis of a more firm and consistent approach towards preventing new introductions.

3.2 PREVENTIVE ACTIONS

Which preventive measures have been identified to reduce the risk of introduction of the organism? Do they reduce the risk to an acceptable level and are they considered as cost-effective? Specify if the proposed measures have undesirable social or environmental consequences. Consider especially (i) the restrictions on importation and trade and (ii) the use of specific holding conditions and effect of prohibition of organism introduction into the wild.

(i) Prohibition of organism importation, trade and holding

At current, accidental importation (e.g. through fish stocking) and natural or secondary translocations are likely to be more important pathways of new introductions in Belgium than escape from farming facilities or trade. Although internet trade for keeping bullfrogs as a pet and illegal pet trade cannot be ruled out, it is probably not significant as the interest in these animals is low. The prevention of secondary translocation of animals should therefore be considered a priority. In this respect, it is urgent to include not only a ban on importation, but also an interdiction of translocation between areas within the non-native range. Moreover, since American bullfrog is established in a cross-border context, Belgium has a clear responsibility to prevent natural spread or translocation of the organism to this neighbouring country. A legal basis for prevention of deliberate release can complement preventive actions and form the basis of a more firm approach towards the prevention of new introductions. It would also render the current legislation consistent with the 1997 ban on introduction of the species in the EU (cf. Scalera, 2007).

(ii) Use of specific holding conditions and effect of prohibition of organism introduction into the wild

NOT APPLICABLE for holding conditions as the species can always escape form garden ponds.

In Flanders, deliberate introduction into the wild is forbidden through the species Decree (Flemish Government Decision of 15 May 2009 on species protection and species management). In the Brussels Capital Region, intentional introduction into nature is already prohibited, as the species is listed as an invasive alien on Annex IV of the recently approved Ordinance on Nature Conservation (March 1, 2012, Article 77). It is also prohibited to sell, hand over (free or with payment), to exchange or purchase American bullfrog. The ordinance also provides the legal basis for preventive actions, control and eradication, for which an advice of the Council for the Environment and the Brussels High Council for Nature Conservation (Art. 78) is required. In the Walloon region, a general interdiction on releasing species into the wild is in force with the Decree on Nature Conservation. However, despite these regulations being in force, new introductions prove hard to avoid and still occur.

3.3 CONTROL AND ERADICATION ACTIONS

Which management measures have been identified to reduce the risk of introduction of the organism? Do they reduce the risk to an acceptable level and are they considered as cost-effective? Specify if the proposed measures have undesirable social or environmental consequences. Consider especially the following questions.

(i) Can the species be easily detected at early stages of invasion (early detection)?

Bullfrog can start a population from only a few fertile females (see higher) (Ficetola et al., 2008a). Therefore, early eradication of propagules is key to prevent further breeding. However, the species is easily overlooked and the very low number of founders (below detection threshold) needed for establishment can hamper efficient detection. Despite this, bullfrog is an emblematic species and experience with an early warning initiative in the border region with The Netherlands has shown that enhanced media attention and training of field workers can be productive in discovering new populations (Devisscher et al., 2012). A further investment in early warning and supporting actions on formation of field workers is therefore justified. Early detection or surveys of bullfrog can be enhanced using environmental DNA (e-DNA) (Valentini et al., 2009). The technique is based on the amplification of DNA released into the environment through mucus, faeces, urine and remains and allows for detection even at very low bullfrog densities (Dejean et al., 2012; Ficetola et al., 2008b).

(ii) Are there some best practices available for organism local eradication?

For an overview of different methods with their pros and cons, as well as a detailed scenario for choosing the best method in the Belgian context, we refer to the framework developed by Devisscher et al. (2012) within the EU funded Interreg project Invexo. These scenarios focus mainly on (1) active trapping using double fyke nets for several consecutive years and (2) drainage of breeding ponds (Figure 13) (which should sometimes also been repeated several times), both with bio-manipulation as potential aftercare.

Figure 13: Removal of remaining tadpoles and adult American bullfrog by seine netting, after the water level of this pond was reduced. When draining the pond a 8-10mm biofilter was put in front of the pump hose in order to prevent any tadpoles from passing the turbid water pump alive. This artificial pond was subsequently filled up again and the parcel restored to acidophilous oak wood (© Invexo).

Other active management methods include frogging (using rifles, nightlighting, gigs, electrofishing, multicapture traps), gillnetting, destruction of egg masses (e.g. Snow & Witmer, 2010, 2011). Chemical methods, such as spraying caffeine on bullfrogs or the use of chloroxylenol or rotenone are difficult to apply in Belgium because of regulations regarding the use of biocides in aquatic environments (cf. 2013 Decree on sustainable use of pesticides in the Flemish Region).

(iii) Do eradication and control actions cause undesirable consequences on non-target species and on ecosystem services?

The use of double fyke nets (Figure 14) allows for replacement of (fish) bycatch in good condition. Therefore, this method is considered animal-friendly and has very few negative non-target effects. The killing of caught animals by overdosing with a benzocaine (ethyl aminobenzoate) solution, MS-222 (tricaine methanesulfonate) or clove oil is considered humane, fast, efficient and harmless for the environment (Close et al., 1996a,b). By preference and for reasons of user-friendliness, the euthanasia is performed in a separate closed container, thereby avoiding any chemical release into the environment.

Adjusting the hydroperiod (hydro-period) of breeding ponds through e.g. short-term drainage or partial filling of ponds to encourage temporary drying out in summer, or - as an emergency measure – the removal or complete conversion of the existing habitat, are clearly management measures with potential effects on other biota. Moreover, when modifications of the water regime are performed, the entire mosaic of suitable habitat in the area should be considered, as these management measures can induce dispersal of juvenile and adult stages into the wider environment. When drainage is performed, it is advisable to fence the area in order to intercept dispersing individuals. In addition to a decline in the bullfrog population, it may be expected that indigenous amphibian populations might equally suffer population losses, especially when draining is performed during the mating season. Thus, the timing of actions is crucial to reduce the non-target effect on native amphibians. The breeding season differs between species, but in Belgium, in general, drainage can best be performed between September and January, after metamorphosis and before the start of the new breeding season of native species. The impact of such measures on hibernating native amphibians is, however, unknown. For more detailed accounts of how to reduce the non-target effects of hydroperiod adjustment we refer to Devisscher *et al*. (2012).

Figure 14: Active trapping of American bullfrog using double fyke nets. This catching gear is relatively cheap, easy to handle and has documented catchability for both larval and adult stages, thereby offering some perspectives for integrated control of populations. Before using these nets in other ponds, disinfection of wading suits with a broad spectrum fungicide and drying of capture gear in the sun for at least three hours are recommended to prevent *Bd* transmission (© Domin Dalessi).

Moreover, if management follows a more holistic approach, integrating active removal with habitat restoration measures, e.g. through the simultaneous removal of invasive fish species or bio-manipulation by introduction of native predatory fish, this is likely to increase the general quality and ecosystem services of the aquatic habitats involved (Louette, 2012; Louette et al., 2012c,d).

(iv) Could the species be effectively eradicated at early stage of invasion?

The species can effectively be eradicated when immediate action is performed after detection. Drastic measures such as active trapping, drainage or complete removal (filling of water body) of a reproduction site can be necessary. The choice of management measures should be based on local habitat conditions, potential non-target effects, sustainability and cost-efficiency.

(v) If widely spread, can the species be easily contained in a given area or limited under an acceptable population level?

When a population has already colonized several breeding ponds (> 50) over large distances (> 10 km), with relatively few dispersal barriers present (e.g. river valleys), complete removal seems unfeasible. Habitat restoration might be an option to mitigate the impact of American bullfrog presence in these cases, where the suitability of the reproduction sites is decreased (e.g. through bio-manipulation and introduction of predators) (Louette, 2012). Further spread along the edges of the infested area can be prevented by active control measures (e.g. double fyke nets) in these water bodies. Such measures should be the subject of a detailed management plan for the complete area, considering key breeding sites, potential dispersal, priority sites for conservation etc.

RISK MANAGEMENT

The relative importance of accidental importation (e.g. through fish stocking) versus natural dispersal or secondary translocations is unknown. Accidental importation of bullfrog larvae hitchhiking in fish stocking lots for angling, is also a pathway to consider. The extent of this phenomenon is however unknown. As there are no reports on farming facilities in Belgium, these pathways are likely to be more important than escapes from rearing facilities. Data on trade of American bullfrog (e.g. in pet shops, on internet) are lacking. In light of these uncertainties, the precautionary principle seems an appropriate way to reduce the risk of both deliberate and accidental importations of American bullfrog in Belgium. The species can easily be overlooked at early stages of invasion. Small, isolated populations can be removed but large interconnected metapopulations are extremely hard to tackle and eradication or control is probably only feasible at very high cost here. Therefore, prevention of further spread, a targeted action plan for existing populations and a dedicated early warning are crucial in tackling this invasive alien in Belgium.

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