Information on measures and related costs in relation to species included on the Union list - *Lithobates catesbeianus*

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Date of completion: 30/05/2019

Comments which could support improvement of this document are welcome. Please send your comments by e-mail to ENV-IAS@ec.europa.eu.

Species (scientific name)	*Lithobates catesbeianus* (Shaw, 1802)
Species (common name)	EN: American bullfrog; NL: Amerikaanse stierkikker, Amerikaanse brulkikker, stierkikker; FR: Grenouille taureau, ouaouaron, grenouille mugissante; SWE: Oxgroda; ES: Rana toro; DE: Nordamerikanische Ochsenfrosch; PT: Rã-touro-americana
Author(s)	Tim Adriaens, INBO – Research Institute of Nature and Forest, Belgium Rein Brys, INBO, Belgium David Halfmaerten, INBO, Belgium Sander Devisscher, INBO, Belgium
Date Completed	30/05/2019
Reviewer	Riccardo Scalera, IUCN Invasive Species Specialist Group & Technical Advisor to the project, Italy

Summary

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures

Methods to achieve prevention

Potential introduction pathways for American bullfrog include escape or release from captivity, deliberate introductions of frogs e.g. to complement the ecosystem, for food and ornamental purposes (e.g. garden ponds) or accidental introductions of larvae through contaminated fish lots. As the EU Wildlife Trade Regulation (338/97) Appendix B already prohibits import of the species into the EU since December 1997, and with the species listed on the list of Union Concern sensu the invasive alien species Regulation (EU 1143/2014), the risk of introducing American bullfrogs is probably low compared to other introduction pathways. Preventive measures should therefore focus on preventing natural colonisation and spread from existing populations and preventing accidental introductions of larvae as stowaways in contaminated fish lots. To prevent unaided spread and colonisation of new habitat, fencing is effectively used to limit the movements and dispersal of amphibians and may play a role in limiting dispersal or protecting sites in specific circumstances. Also, the prevention (discouragement) of new habitat creation around existing (meta)populations might be a simple yet controversial method to prevent colonisation. The fish lot pathway should be subjected to increased inspections and control. Furthermore, awareness raising can be performed with fishermen and garden pond owners about the risk of accidentally transport larvae when moving fish. Also, awareness raising to prevent further introductions and spread of animals released for food purposes is needed.

Surveillance measures to support early detection

The species can easily be overlooked at early stages of invasion, which warrants the application of environmental DNA (eDNA) in complement of classic surveys for early detection. The eDNA 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 population densities. Also, it is known that populations can be present and remain unnoticed for a long time in an area. Around existing populations, dedicated citizen science surveys can be organised, where people are trained to recognise and detect bullfrog e.g. by learning to identify American bullfrog adults and larvae, and to recognise the sound of adult and juvenile bullfrogs, reproductive calls and alarm calls. A possibility is also to use waveforms and sonograms for bullfrog identification, based on high quality digital sound recording. The use of acoustic validation has been effective to make the most from data from citizen science in a national survey for frogs in Australia. Volunteers can then adopt a certain area assigned to them, perform door-to-door interviews with garden pond owners and perform inspections of garden ponds. Also, interviews with anglers can be an effective method to learn about bullfrog presence. In some cases, hand netting and eDNA sampling events can be conducted with the help of volunteers as well, provided they are trained to take appropriate water samples. Sniffer dogs can also be trained and used to detect American bullfrog, e.g. in overwintering sites, but this is more useful for detecting bullfrog in known populations. Raising awareness of the problems posed by the release or presence of American bullfrog, and invasive alien species in general, can reduce the risk of further escapes and can foster rapid reporting of new populations to support a rapid response. As American bullfrogs are popular with the press, media attention can be used to increase awareness and involve the general public (e.g. garden pond owners) in early detection.

Methods to achieve eradication

The species can effectively be eradicated when immediate action is performed after detection. Drastic measures such as a pond destruction or a

combination of drainage and active trapping are necessary to achieve this, but there are many documented cases of effective eradication. The site should be fenced during intervention to prevent bullfrog dispersal due to changes in the hydro-regime. The choice of management measures should be based on local habitat conditions, potential non-target effects on native wildlife (other amphibians, fish, freshwater invertebrates, etc.), sustainability and cost-efficiency. Small, isolated populations can be removed, but large interconnected meta-populations are extremely hard to tackle and eradication or control is probably only feasible at very high cost and with appropriate political and stakeholder support alongside dedicated budget lines.

Methods to achieve control and mitigate impact

Effective control of bullfrogs requires tackling the species at all developmental stages (larvae, metamorphs, adults) simultaneously. The choice of a management measure is a trade-off between the ecological preconditions of the habitat, the desired conservation goals, any economic constraints and the relative importance assigned to different criteria of management feasibility (practicality, cost-efficiency, non-target effects). Population models indicate that removal of larval stages is less effective in affecting population growth rates than removal of post-metamorphic stages. However, the removal of larvae is much more practical due to their limited mobility and because they are entirely aquatic. To control American bullfrogs, a combination of methods such as active trapping (e.g. using fyke nets, seine netting) or shooting can be used. Other active management methods include frogging (using rifles, nightlighting, gigs, electrofishing, multi-capture traps), gillnetting and destruction of egg masses. Fencing is recommended when any control operations are carried out on water bodies, such as adjusting the hydroperiod or whilst performing drainage, pond destruction, seine netting, fyke netting, electrofishing etc., as these measures tend to induce bullfrog dispersal. Under such conditions, bullfrog often shows movements away from sites undergoing control, and fencing may enhance removal operations success and reduce the chance of further colonisation. Chemical methods, such as spraying caffeine on bullfrogs, liming, the use of chloroxylenol or rotenone are probably more difficult to apply because of regulations regarding the use of biocides in aquatic environments, animal welfare concerns and increased non-target effects on other biota. Exotic fish species such as non-native pumpkinseed *Lepomis gibbosus* and topmouth gugdeon *Pseudorasbora parva* (another Union List species) can facilitate American bullfrog by their predation on macroinvertebrates that feed on tadpoles. Therefore, when controlling American bullfrogs, it is preferable to also remove those non-native fish. Habitat restoration (e.g. through bio-manipulation and introduction of native predators) might be an option to mitigate the impact of American bullfrog presence, where the suitability of the reproduction sites is decreased. This method can also be used in complement or as aftercare in controlled populations. Although not empirically tested yet, it is expected that the release of sterile triploid males may also have a significant and negative impact on population growth rates, especially in small or newly colonised areas. However, this release of sterile males is still being tested and further research into its impact on bullfrog populations is required. Any measures in large populations should be the subject of a detailed management plan for the complete area, considering key breeding sites, potential dispersal routes, priority sites for conservation etc.

Prevention of <u>intentional</u> introductions and spread – measures for preventing the species being introduced intentionally. **This table is repeated for each of the prevention measures identified.** *If the species is listed as an invasive alien species of Union concern, this table is not needed, as the measure applies anyway*

Measure description Provide a description of the measure, and identify its objective	*As the species is listed as an invasive alien species of Union concern, the following measures will automatically apply, in accordance with Article 7 of the EU IAS Regulation 1143/2014:* *Invasive alien species of Union concern shall not be intentionally:* *(a) brought into the territory of the Union, including transit under customs supervision;* *(b) kept, including in contained holding;* *(c) bred, including in contained holding;* *(d) transported to, from or within the Union, except for the transportation of species to facilities in the context of eradication;* *(e) placed on the market;* *(f) used or exchanged;* *(g) permitted to reproduce, grown or cultivated, including in contained holding; or* *(h) released into the environment.* *Also note that, in accordance with Article 15(1) – As of 2 January 2016, Member States should have in place fully functioning structures to carry out the official controls necessary to prevent the intentional introduction into the Union of invasive alien species of Union concern. Those official controls shall apply to the categories of goods falling within the Combined Nomenclature codes to which a reference is made in the Union list, pursuant to Article 4(5).]* ***Therefore measures for the prevention of intentional introductions do not need to be discussed further in this technical note.***

Prevention of <u>un-intentional</u> introductions and spread – measures for preventing the species being introduced un-intentionally (cf. Article 13 of the IAS Regulation). **This table is repeated for each of the prevention measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Increased biosecurity.** Control for pathways of unintentional introduction and spread can be performed through increased biosecurity measures to prevent new introductions. American bullfrog larvae can be introduced as stowaways in consignments of fish for stocking or for aquaculture. Especially fish used for stocking waters and rivers can be an important pathway for unintentional introduction. Lots with ornamental fish (goldfish, koi) are expected to be less risky, as they are normally sorted before going to retailers (Adriaens et al. 2013), although reports of larvae hitchhiking as stowaways in lots of golf fish *Carassius auratus* exist e.g. in Spain (Cabana & Fernández 2010). Contamination of fish lots with tadpoles can happen if fish are temporarily stocked on fish ponds before shipment. This is also an important pathway for other aquatic non-natives, including Union List species such as topmouth gudgeon *Pseudorasbora parva* (Verreycken et al. 2007; Copp et al. 2010) or Amur sleeper *Perccottus glenii* (Verreycken 2013; Verreycken et al. 2007, 2009). Checking for stowaway specimens of American bullfrog (and other invasive alien species) in fish farms and fish transports from within (and outside) the EU is a potential method for preventing new accidental introductions. However, the extent to which this is happening in Europe is largely unknown and possibly natural colonisation and spread from existing populations is a much more important pathway of introduction. Most of the fish used for stocking in western European regions originates from eastern Europe (pers. comm. H. Verreycken). American bullfrog is thought to be absent there, but contamination of these lots potentially happens at stop-over ponds. Fish transports should be regularly checked for stowaways. Import control of large trucks for fish transport, however, has proved to be very difficult. Also, rigorous control actions are needed when fish stocking activities in public waters are executed. The checking of fish transport for stowaways can be done manually/visually (with a dip-net) or via new techniques such as eDNA. Samples from the consignment can be visually checked for non-wanted species, however this is very difficult when the stowaway species is present in small quantities or when the container to be checked is very big, so unwanted species can be easily overlooked (pers. comm. Hugo Verreycken). New tools like environmental DNA (eDNA) are promising. Water samples should be taken conform the procedure, and in the laboratory the water samples should be analysed. Depending on the technique used, one can test for the presence of one species, several unwanted species or the whole array of fish present in the containment. Unfortunately, at the moment, these tests are not quick enough to be used as an instant tool to detect stowaways. Temporary quarantine of newly arrived fish consignments is necessary to wait for the results (at least 48h). Apart from increased inspections and control of fish lots, awareness raising can be performed with fishermen, aquaculturists and garden pond owners about the risk of accidental translocation of larvae when moving fish or

<table>
<tr><td></td><td>other biota.</td></tr>
<tr><td>Scale of application
At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.</td><td>No data are available on whether this measure has been applied yet or about the scale this measure has been applied at in Europe nor anywhere else. Most Member States have some form of preventive measures to avoid unintentional introductions of stowaway species in consignments (e.g. Live Fish Act in the UK), but it is unclear how and whether they are enforced. This measure to screen fish consignments should be applied for fish transports at points of entry into both the EU and the Member States, particularly where the species is not present yet.</td></tr>
<tr><td>Effectiveness of the measure
Is it effective in relation to its objective? Has the measure previously worked, failed?

Please select one of the categories of effectiveness (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td><td><table><tr><td>Effectiveness of measures</td><td>Effective</td><td></td><td>Neutral</td><td></td><td>Ineffective</td><td>X</td><td>Unknown</td><td></td></tr></table>
Rationale:
Manual and visual checking of fish transports for stowaways is difficult and unwanted species can easily be overlooked. Therefore, the screening of fish consignments is not fully efficient (Copp et al. 2010). New tools like environmental DNA (eDNA) are promising, but at the moment these tests are not quick enough to be used as an instant tool to detect stowaways (pers. comm. J. Mergeay). Testing for eDNA of unwanted species is ideally combined with temporary quarantine of newly arrived fish consignments. In the future, this measure may become more effective through the use of these new molecular techniques.</td></tr>
<tr><td>Effort required
e.g. period of time over which measure needs to be applied to have results</td><td>In order to be effective, biosecurity should be applied consistently at all levels of organisation (Caffrey et al. 2014). These measures should be taken any time fish transports are due to happen. On a yearly base, for traditional aquaculture of coldwater fish, this would mainly occur in spring and autumn. Only when a (law enforced) system is developed, where all fish transports are reported, these measures could be more effective, but it would still need flexible authorities to effectively check all transports.</td></tr>
<tr><td>Resources required [1]
e.g. cost, staff, equipment etc.</td><td>The resources needed for this preventive measure include: staff costs, costs for cars and material to check the consignments, budgets for performing DNA tests and quarantine facilities. Costs can be reduced when samples of consignments are taken at the fish farm rather than at the stocking sites (public, as well as private waters) because the fish farms are less numerous than the number of stocking sites and, when quarantine is necessary, this can be done at the fish farm. Problems with access to private lands may hinder this last option, depending on whether Member State legislation provides for this. No published information is available about the required resources for these measures.</td></tr>
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Side effects (incl. potential) – both positive and negative i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc. For each of the side effect types please select one of the impact categories (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Environmental effects***</td><td>*Positive*</td><td>*X*</td><td>*Neutral or mixed*</td><td></td><td>*Negative*</td><td></td></tr><tr><td>***Social effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td></td><td>*Negative*</td><td>*X*</td></tr><tr><td>***Economic effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td></td><td>*Negative*</td><td>*X*</td></tr></table> *Rationale*: A positive environmental effect could be the possible detection of other, non-desired invasive species that are known to be introduced through the same pathways, such as topmouth gudgeon *Pseudorasbora parva* (Copp et al. 2010) or Amur sleeper *Perccottus glenii* (Verreycken 2013), both Union List species. A negative social effect could be that fish consignments would not be stocked because consignments are destroyed, fish died in quarantine or because of delays in restocking. A negative economic effect could occur for the fish farmer when an infested consignment is found. The effect could become bigger if the infestation was detected at the level of an entire fish farm. Proper sorting of the fish specimens before loading a consignment will also take longer, and this can also be considered as a small negative economic effect.
Acceptability to stakeholders e.g. impacted economic activities, animal welfare considerations, public perception, etc. Please select one of the categories of acceptability (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Acceptability to stakeholders***</td><td>*Acceptable*</td><td></td><td>*Neutral or mixed*</td><td>*X*</td><td>*Unacceptable*</td><td></td></tr></table> *Rationale*: Acceptability can be mixed. In some fish ponds, fish farmers may be happy to remove American bullfrog, as their tadpoles may compete with, or their adults predate on, farmed fish. Others might complain about non-stocked fish loads and more work to properly sort out the fish specimens. As the sorting out of the fish will need to be more precise, it will also take longer, which eventually may also lead to higher mortalities in the stocked fish consignments.
Additional cost information [1] When not already included above, or in the species Risk Assessment. - implementation cost for Member States - the cost of inaction - the cost-effectiveness - the socio-economic aspects	No information available.

Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Inconclusive* ☐ *Unresolved* ☐ *Established but incomplete* X *Well established* ☐ *Rationale*: Very little information about the control of fish consignments is available, but most studies agree that stowaways in fish consignments are a major introduction pathway which should be controlled.

Prevention of <u>secondary spread</u> of the species – measures for preventing the species spreading once they have been introduced (cf. Article 13 of the IAS Regulation). **This table is repeated for each of the prevention measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Physical dispersal barriers.** Prevention of unaided secondary spread can be achieved through installing physical dispersal barriers (fencing). The aim of fencing (also referred to as amphibian fencing, amphibian exclusion fencing, temporary amphibian fencing, drift fencing, barrier fencing) is to prevent further dispersal of American bullfrog from already existing populations into new suitable reproduction habitats (ponds, permanent water bodies). This measure can be especially useful to restrict dispersal of newly introduced populations, to protect vulnerable sites to bullfrog invasion, and to limit movements away from sites undergoing control. American bullfrogs make several migration movements throughout the year (Devisscher et al. 2012; Descamps & De Vocht 2016). In spring and late fall the adults migrate from wintering habitats to their breeding ponds and vice versa, and throughout the summer juveniles try to escape cannibalism by fleeing the breeding ponds and moving towards suboptimal or new, not yet invaded habitats (e.g. shallow ponds, ditches, etc.). Also, it is known that dispersal events mostly occur when population densities exceed certain thresholds (Moissonier et al. 2007). Fencing prevents American bullfrogs from using dispersal corridors/routes and is an effective method to limit further spread from existing populations. When amphibian fences are combined with trapping using buckets, this may also reduce densities of post-metamorphic life stages.

	Besides migration events over land, ponds are often also connected via ditches or rivers, or via overflows or frequent flooding, which may also facilitate the spread of American bullfrog larvae. Such connections should be closed or modified by placing, for instance, a fine mesh (<8mm) grid to limit further spread. Natural dispersal rates of American bullfrog can be considerable, from several hundred meters up to several kilometres (Descamps & De Vocht 2017), which has for instance been documented in several European populations in Flanders (Adriaens et al. 2013) and France (Ficetola et al. 2007). Post-metamorphic stages are capable of dispersing long distances and typically colonise new sites (> 1,200 m) (Willis et al. 1956). To escape predation by larger conspecifics, juveniles often migrate shortly after metamorphosis to suboptimal habitats, such as smaller, sometimes heavily shaded ponds, where few or no adult bullfrogs are present. For instance, Jooris (2005) documented that, in the Great Nete valley in Belgium, juveniles concentrated in ditches along meadows, in riparian vegetation and in marshy areas to avoid direct contact with adult individuals. Adult American bullfrogs, on the other hand, are known for their relative good dispersal capacity over distances of more than three kilometres (Stoutamire 1932; Lougheed & Taylor 2010). Data inferred from reproducing populations (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 (Adriaens et al. 2013). Maximum distances recorded with capture mark recapture studies of adult individuals are 1,600, 914 and 966 meters (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). 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). Fencing can be an efficient management strategy to **prevent dispersal of adults and metamorphs**, which actively disperse out of their reproductive habitat. To be effective, fencing for American bullfrog requires a (plastic or mesh) amphibian screen at least 1 m high to be placed around the pond (Damen 2010; Herder et al. 2013). Such a screen ideally needs to be dug into the ground about 10 cm deep. To collect and eradicate dispersing American bullfrog individuals, buckets (> 10 l) can be buried, closed with a lid, but with a 10 cm diameter hole. To prevent the buckets from filling up with water, it is best to provide them with holes (1 cm) at the bottom, so that excess water can drain away into the soil (this is only applicable if the groundwater level remains below the bottom of the bucket). For animal welfare reasons and to avoid side effects on other biota, especially other amphibian species, it is necessary to check the buckets frequently (every 24-48 hours) and release any native bycatch (Michelin 2012; Sarat et al. 2016; Devisscher et al. 2017). To **prevent larval dispersal**, for instance from a pond to a connected waterbody such as rivers, streams or brook forest, other means of physical barriers (protective screens, biofilters) are needed, suitable to closing inlets, outlets or overflows. When drainage of ponds is performed, care should be taken to install an appropriate biofilter to

<table>
<tr><td></td><td>prevent any live larvae to be pumped out of the water into another waterbody. For example, during the Invexo Interreg project, a 1,000m^2 pond (estimated volume 1,500 m^3) was drained with a 40 m^3/h flow rate pump. An 8 mm filter was installed on the suction head, assuming smaller tadpoles would not survive the operation (Devisscher et al. 2012). Invaded ponds that frequently overflow need to be additionally protected, for instance by installing dykes or increasing the height of the pond edges with sediment, to avoid further spread of larvae.</td></tr>
<tr><td>Scale of application
At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.</td><td>Fencing is suitable for small (< 4000 m^2) ponds and ponds with little or no vegetation along the bank. Other physical barriers (biofilters, closure of inlets) potentially can prevent larval dispersal out of much bigger water bodies.</td></tr>
<tr><td>Effectiveness of the measure
Is it effective in relation to its objective? Has the measure previously worked, failed?

Please select one of the categories of effectiveness (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td><td><table><tr><td>Effectiveness of measures</td><td>Effective</td><td>X</td><td>Neutral</td><td></td><td>Ineffective</td><td></td><td>Unknown</td><td></td></tr></table>
Rationale:
The method is well established and has been widely applied in Europe and elsewhere for frogs, toads and newts. For instance, removal of a population in a garden pond in the Netherlands was successfully achieved using a combination of fencing, draining and active trapping (Creemers 2011a,b).</td></tr>
<tr><td>Effort required
e.g. period of time over which measure needs to be applied to have results</td><td>The fence or dispersal barrier should be installed as long as there is a risk for bullfrogs dispersing out of the invaded habitat. The integrity of the fence should be regularly checked (vandalism, falling branches). If a fence is kept for several consecutive years, appropriate materials should be chosen that resist UV degradation (e.g. permanent exclusion panels made from UV stable recycled plastic).</td></tr>
<tr><td>Resources required [1]
e.g. cost, staff, equipment etc.</td><td>There are significant differences in the cost associated with the type of dispersal barrier used. The closing of non-permanent inlets and outlets is a simple and inexpensive measure. Adjusting permanent overflows and dikes entails a considerable one-off cost. Fencing is relatively cheap in terms of the required materials and work effort for the setup, but when combined with passive trapping, checking the buckets for captured bullfrogs requires high effort in terms of time investment. However, this cost can be reduced by using volunteers.
The price of amphibian fencing is highly dependent on site conditions. Although many suppliers of amphibian fences quote prices on a per metre installed basis, site access, ground conditions, presence of vegetation, tree roots and site security can cause considerable variation in price. Also, the type of material causes differences in price. For</td></tr>
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<td></td>
<td>example, a standard 100 m x 1000 mm roll of polythene newt fence is priced at 60-100€, whereas permanent panels can cost 30€ per 2.5 x 0.85 m panel (www.wildcare.co.uk, www.wildlifefencing.co.uk). The price of poles, stakes, screws and waterproof repair tape should be added. In the case of large ponds and/or more vegetated banks, the cost of fencing will increase. Screens can be sensitive to vandalism, for example along footpaths or accessible sites, potentially incurring added costs.</td>
</tr>
<tr>
<td>Side effects (incl. potential) – both positive and negative
i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc.

For each of the side effect types please select one of the impact categories (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td>
<td>
<table>
<tr><td>Environmental effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td></td><td>Negative</td><td>X</td></tr>
<tr><td>Social effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
<tr><td>Economic effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
</table>

Rationale:
The non-target environmental effects of fencing are negative, as the presence of a fence also prevents dispersal of native species both to and from a site. This effect will vary depending on the timing of application and the period during which the waterbody is fenced. No social or economic effects of fencing are documented.</td>
</tr>
<tr>
<td>Acceptability to stakeholders
e.g. impacted economic activities, animal welfare considerations, public perception, etc.

Please select one of the categories of acceptability (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td>
<td>
<table>
<tr><td>Acceptability to stakeholders</td><td>Acceptable</td><td></td><td>Neutral or mixed</td><td>X</td><td>Unacceptable</td><td></td></tr>
</table>

Rationale:
The prevention of dispersal of native amphibians to and from a fenced site might be unacceptable to conservationists. However, this effect can be mitigated using buckets to retrieve dispersing amphibians to and from the site, followed by translocation, and also depends on the timing and duration of keeping the barriers installed.</td>
</tr>
<tr>
<td>Additional cost information [1]
When not already included above, or in the species Risk Assessment.
- implementation cost for Member States
- the cost of inaction
- the cost-effectiveness
- the socio-economic aspects</td>
<td>No information available.</td>
</tr>
</table>

Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	<table><tr><td>*Inconclusive*</td><td></td><td>*Unresolved*</td><td></td><td>*Established but incomplete*</td><td></td><td>*Well established*</td><td>*X*</td></tr></table> *Rationale*: Fencing is a well-established method that has been widely used to prevent amphibian dispersal.

Prevention of secondary spread of the species – measures for preventing the species spreading once they have been introduced (cf. Article 13 of the IAS Regulation). **This table is repeated for each of the prevention measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Discouragement of the creation of new habitat.** Prevention of unaided secondary spread can be achieved through discouragement of the creation of new habitat in the vicinity of existing bullfrog populations. This involves refraining from digging ponds and creating aquatic habitat in a buffer zone around existing populations. In practice, this can be achieved through the refusal of permits for creating ponds.
Scale of application At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	Considering American bullfrog dispersal capacity, creation of new ponds or wetlands should be avoided in a buffer zone of 3 km around existing populations, to prevent further spread (Descamps & De Vocht 2017).

Effectiveness of the measure Is it effective in relation to its objective? Has the measure previously worked, failed? Please select one of the categories of effectiveness (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Effectiveness of measures***</td><td>*Effective*</td><td></td><td>*Neutral*</td><td></td><td>*Ineffective*</td><td></td><td>*Unknown*</td><td>*X*</td></tr></table> *Rationale*: To our knowledge, there are no documented examples of this method available. The method has been proposed in Belgium around existing bullfrog strongholds, but documentation is lacking.
Effort required e.g. period of time over which measure needs to be applied to have results	The method would have to be applied as long as there are populations of American bullfrog present in an area.
Resources required [1] e.g. cost, staff, equipment etc.	In principle the method is cheap, as it involves the refusal of a permit for creating wetland habitat.
Side effects (incl. potential) – both positive and negative i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc. For each of the side effect types please select one of the impact categories (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Environmental effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td></td><td>*Negative*</td><td>*X*</td></tr><tr><td>***Social effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td></td><td>*Negative*</td><td>*X*</td></tr><tr><td>***Economic effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td></td><td>*Negative*</td><td>*X*</td></tr></table> *Rationale*: There are no documented examples of this method available, but as the creation of pond habitat is a classic conservation measure for amphibians and other biota, environmental effects on other biota are negative. Also, this method can invoke negative reactions from the public, conservationists and conservation managers, who might not agree that the prevention of American bullfrog dispersal outweighs native species conservation. To not perform a planned project can also represent a loss of economic revenue/interest.
Acceptability to stakeholders e.g. impacted economic activities, animal welfare considerations, public perception, etc. Please select one of the categories of acceptability (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Acceptability to stakeholders***</td><td>*Acceptable*</td><td></td><td>*Neutral or mixed*</td><td>*X*</td><td>*Unacceptable*</td><td></td></tr></table> *Rationale*: The method can invoke negative reactions from the public, conservationists and conservation managers, who might not agree that the prevention of bullfrog dispersal outweighs native species conservation. Also, if ponds are planned for angling purposes, anglers might oppose.

Additional cost information [1] When not already included above, or in the species Risk Assessment. - implementation cost for Member States - the cost of inaction - the cost-effectiveness - the socio-economic aspects Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	No information available.
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Inconclusive* | *X* | *Unresolved* | | *Established but incomplete* | | *Well established* | *Rationale*: To our knowledge, there are no documented examples of the discouragement of the creation of new habitat.

Surveillance measures to support early detection - Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory. **This table is repeated for each of the early detection measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Monitoring with citizen science.** Monitoring with citizen science can offer great support to both general and risk oriented surveillance. In general, the accuracy of the data volunteers collect compares favourably to that of professionals (Crall et al. 2010, 2011, 2015). Using on-line recording and smartphone apps, along with the development of social media, allows for fast dissemination of new records, reduces mistakes and data loss, and improves the speed of record submission, uptake by databases and early warning alerts (Adriaens et al. 2015; August et al. 2015). For Union List species such as

	bullfrog, a number of general reporting portals (e.g. iNaturalist, Natusfera, Observation.org, Artsdatabanken,…) and specific applications (e.g. overview on https://easin.jrc.ec.europa.eu/easin/CitizenScience/About) are available to report occurrences. Motivations of various target groups might differ, therefore citizen science programs should be targeted towards the audience at hand. For American bullfrog, naturalists, herptile enthusiasts, garden pond owners, anglers and also the general public are relevant target groups. In addition, monitoring with citizen science can play a significant role in public engagement, improved education and public awareness about invasive alien species, and is recognised as fundamental to the attainment of the objectives of invasive species policies (Roy et al. 2018). Volunteers focus mainly on visual observations of all American bullfrog life stages, on reproductive calls (the loud mating whrumm-whrumm ("more rum") sound produced by the male and described as the sound of a bull or recalling the sound of a bittern) and emergency calls (shrieking "iep-sound" when juveniles flea). Although the fast squeak of juvenile bullfrogs produced as an alarm call before jumping into the water is easily recognisable, a possibility is also to use (automated) digital sound recording and analysis software to confirm the presence and identify bullfrog based on waveforms and sonograms (e.g. Kime et al. 2000; Duan et al. 2013; RAVEN software Cornell). The use of acoustic validation has been effective to make the most from data from citizen science in a national survey for frogs in Australia (Rowley et al. 2019). The involvement of volunteers can also allow to efficiently upscale surveys e.g. for taking water samples to be analysed with eDNA. On the other hand, misidentifications can lead to unnecessary responses and therefore a waste of resources, so data validation is key. Around existing populations, dedicated citizen science surveys can be organised, where people are trained to recognise and detect the species e.g. by learning to identify adults and larvae of American bullfrog and other confusing species present in the area, and to recognise the sound of adult and juvenile bullfrogs, reproductive calls and alarm calls. Volunteers can then adopt a certain area assigned to them, perform door-to-door interviews with garden pond owners and perform inspections of garden ponds. Also, interviews with anglers can be an effective method to learn about bullfrog presence. As American bullfrog are popular with the press, media attention can be used to increase awareness and involve the general public (e.g. garden pond owners) in early detection. In some cases, hand netting and eDNA sampling events can be conducted with the help of volunteers as well, provided they are trained to take appropriate water samples. Monitoring amphibians, whether by specialist herpetologists or volunteers, has a long tradition and several good protocols are available outlining potential methods, such as visual searches, scanning with binoculars, dip netting, meadow surveys, nocturnal surveys, the use of amphibian fykes and pond dipping (e.g. Fellers & Freel 1995, www.amphibiaweb.org).
Scale of application At what scale is the measure applied? What is the largest scale at which it	Surveillance using citizen scientists can be applied at any desired geographic scale. It is useful to implement general, large scale surveillance to train volunteers in identifying the species and to intercept any solitary escaped or translocated individuals in uninvaded areas. Around existing populations, more dedicated (risk-oriented or aimed at

has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	containing or managing the species) citizen science surveys can be organized. As a case study, this approach was followed in an Interreg project (www.invexo.be) in the border region between Belgium and The Netherlands, to prevent any further cross-border American bullfrog dispersal (Devisscher et al. 2012). An early warning network was set up in a region of over 34 km^2 using existing reporting tools (www.waarnemingen.be and www.waarneming.nl). Interested parties were to follow a training course on recognition of bullfrog (sound, behaviour and appearance). Volunteers adopted specific km squares for inspection on bullfrog presence. Any reports were then checked by experts. The media attention eventually prompted the discovery of a reproducing population in two breeding ponds in Baarlo (Limburg) which, based on interviews with locals, seemed to have been present (and unnoticed) for more than ten years in the area (Creemers 2011a,b). After a thorough inventory was performed (Creemers 2011a,b), the populations in two breeding ponds were promptly removed in 2011-2012 through a combination of fencing, seine netting, fyke netting, electrofishing and drainage. A nearby site was screened for bullfrog presence with environmental DNA techniques (Crombaghs 2012; Goverse et al. 2012).
Effectiveness of the measure Is it effective in relation to its objective? Has the measure previously worked, failed? Please select one of the categories of effectiveness (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	*Effectiveness of measures* — *Effective*: *X*; *Neutral*: ; *Ineffective*: ; *Unknown*: *Rationale*: Examples from the Netherlands and Flanders (Belgium) show that large-scale citizen science programmes can effectively yield occurrences of American bullfrog individuals from populations that were previously unknown (Devisscher et al. 2012; Vanderhoeven et al. 2015).
Effort required e.g. period of time over which measure needs to be applied to have results	Surveys are best performed in the period May-September. Calling males can be heard in the period April-August. Juvenile and subadults can be heard from April to October. Surfacing larvae can be seen in summer (June-August) by scanning the water surface.
Resources required [1] e.g. cost, staff, equipment etc.	The cost incurred when running citizen science projects includes human resources for coordination, communication and for providing training and feedback to volunteers, set-up and updates of e-infrastructure, recording tools or sampling kits (apps, eDNA kits etc.), and the production of identification sheets, leaflets etc. The cost of setting up a smartphone app, for instance, can greatly vary depending on the target species (groups) and its functionalities (Adriaens et al. 2015). However, one should be aware that running a proper citizen science survey is more than launching an app, website or tool, and does incur costs for keeping the volunteer network running.
Side effects (incl. potential) –	

both positive and negative i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc. For each of the side effect types please select one of the impact categories (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Environmental effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td>*X*</td><td>*Negative*</td><td></td></tr><tr><td>***Social effects***</td><td>*Positive*</td><td>*X*</td><td>*Neutral or mixed*</td><td></td><td>*Negative*</td><td></td></tr><tr><td>***Economic effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td>*X*</td><td>*Negative*</td><td></td></tr></table> *Rationale*: Surveillance using visual and auditory surveys, or interviews with pond owners or anglers, represent non-invasive methods. It is anticipated that they incur no notable non-target effects. Movement of volunteers between water bodies represents a biosecurity issue. Care should be taken to avoid the spread of invasive and/or pest species such as New Zealand pygmyweed *Crassula helmsii*, Nuttall's pondweed *Elodea nuttallii,* Canadian waterweed *E. Canadensis,* curly waterweed *Lagarosiphon major,* water Fern *Azolla filiculoides,* flowering water primrose *Ludwigia grandiflora,* floating pennywort *Hydrocotyle ranunculoides* or parrot's feather *Myriophyllum aquaticum*. Also, it is thought that field staff could act as potential vectors of transmission of amphibian diseases into new sites and to naïve species. The risk of this mode of transmission is not fully understood compared to other vectors such as waterfowl and other wildlife. However, as a precaution, it is essential that field workers follow hygiene protocols to prevent further spread of amphibian disease such as Ranavirus or chytrid fungus *Bd* and *Bsal*. Several hygiene protocols are available (check, clean, dry) (e.g. Froglife 2016, RAVON 2017). A positive environmental effect of citizen science might be the identification and recording of other IAS, as well as an increased awareness on IAS. Being outdoors is also known to be a prime motivator for people to engage in citizen science programmes, hence a positive social effect.
Acceptability to stakeholders e.g. impacted economic activities, animal welfare considerations, public perception, etc. Please select one of the categories of acceptability (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Acceptability to stakeholders***</td><td>*Acceptable*</td><td></td><td>*Neutral or mixed*</td><td>*X*</td><td>*Unacceptable*</td><td></td></tr></table> *Rationale*: As the method is based on visual/auditory observations, it is generally non-invasive. There might be issues with access to private land or inaccessible nature reserves by volunteers. Reporting through citizen science (e.g. in private garden ponds) can potentially result in management actions being undertaken, which owners do not always agree with.
Additional cost information [1] When not already included above, or in the species Risk Assessment. - implementation cost for Member States	No information available.

- the cost of inaction - the cost-effectiveness - the socio-economic aspects Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Inconclusive* ☐ *Unresolved* ☐ *Established but incomplete* X *Well established* ☐ *Rationale*: The use of volunteers for bullfrog surveillance (and citizen science for IAS reporting in general) is well established, but information on its effectiveness, as compared to other methods, is mostly lacking (e.g. compared to eDNA samplings).

Surveillance measures to support early detection - Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory. **This table is repeated for each of the early detection measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Monitoring with environmental DNA.** To achieve early detection of new founder populations and to obtain a complete overview of the situation when an area is infested by American bullfrog, monitoring with environmental DNA (eDNA) is a cost-effective and efficient approach (Valentini et al. 2009). The technique is based on the amplification of DNA released into the environment through skin mucus, faeces, urine and remains, and allows for detection even at very low bullfrog densities (Ficetola et al. 2008b; Dejean et al. 2012). Also, prior to eradication campaigns, eDNA sampling can offer clear insights on presence/absence of the species in the landscape, and can also offer relative indications on population density (Herder et al. 2013). The technique is also applicable to other non-native aquatic species, such as crayfish, mitten crabs, non-native fish etc. Because this approach can detect the presence of a few DNA molecules in the water, the presence of a species, even at very low densities, can be determined very accurately (Ficetola et al. 2008b; Dejean et al. 2012). To obtain a full-covering overview of an American bullfrog (meta)population at landscape level in a certain area of

	interest, eDNA analyses offer an appropriate tool to screen a large number of sites/ponds at the same time. The obtained insights on presence/absence and relative abundance can be very useful to implement a cost-effective and efficient eradication campaign (e.g. through network analysis of the metapopulation). Also at early stages of invasion, when bullfrog individuals establish at low densities, such satellite (sub)populations can be easily overlooked with traditional monitoring surveys. Since eradication is less costly and most efficient when applied at such early stages of invasion (due to less individuals that need to be removed), eDNA screening is a cost-effective and efficient methodology to obtain a reliable overview of the actual situation (Ficetola et al. 2008a). Prior to eradication campaigns, eDNA sampling can offer clear insights on presence/absence of the species in the landscape, and can also offer relative indications on population density (Herder et al. 2013). eDNA analyses need to be carried out in labs that are able to carry out eDNA analyses following the general guidelines and protocol restrictions, in order to avoid false positive or false negative detections. Additionally, several control mechanisms need to be incorporated, such as the inclusion of field- and lab-blanks (blanco samples), and positive controls with bullfrog eDNA (Harper et al. 2018). Species-specific eDNA approaches, such as qPCR- and ddPCR-methods, are far more sensitive in terms of detection probabilities and also offer better insights in eDNA copy numbers per liter water than the much more generalistic eDNA meta-barcoding approaches. With the development and implementation of new generation PCR approaches, the use of Digital Droplet PCR (ddPCR) analyses is preferred above traditional or quantitative PCR (qPCR) analyses. In comparison to qPCR- analyses, the ddPCR-approach is more sensitive to detect very low eDNA concentrations, is able to estimate exact concentrations, and is also less prone to inhibition at the stage of amplification and thus successful detection. The latter, thus, consequently results in lower rates of false negative detections. To avoid the risk of cross-contamination during the process of water filtering in the field or lab, but also at the stage of eDNA extraction, the use of enclosed capsule filters is generally recommended.
Scale of application At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	Depending on the situation, and taking into account its dispersal capacity and average yearly movement, all ponds in a 1500 m range around a newly discovered bullfrog population - an area of approximately $7km^2$ (Devisscher et al. 2012) - should be sampled and tested for the presence/absence of the species with eDNA. In practise, however, landscape features and man-made barriers (like canals and large roads) can greatly reduce the potential spread of the species, and thus also limit the search area (Jooris 2005). In the Netherlands, near the town of Baarlo, eDNA monitoring was used to assess the full extent of an American bullfrog invasion, to support the eradication campaign (van Delft et al. 2013). The focal area was about 0.6 km^2. Also in the Netherlands, an early warning network was set up near the Belgian border, where bullfrog populations are present, and supplemented by eDNA monitoring. Since 2013, about 30 water bodies have been sampled annually and, until now, no bullfrogs have been detected in this network (van Delft et al. 2018). On a larger scale, a successful eDNA survey was performed in France, in which an area of 25 km^2 around 50 ponds were surveyed (Dejean et al. 2012).

<table>
<tr><td></td><td></td></tr>
<tr><td>Effectiveness of the measure
Is it effective in relation to its objective? Has the measure previously worked, failed?

Please select one of the categories of effectiveness (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td><td><table><tr><td>Effectiveness of measures</td><td>Effective</td><td>X</td><td>Neutral</td><td></td><td>Ineffective</td><td></td><td>Unknown</td><td></td></tr></table>
Rationale:
eDNA methods are more accurate than traditional methods, such as visual surveys. An eDNA survey in France revealed that the occurrence of bullfrog was found to be around five times higher than previously expected based on traditional survey methods (Dejean et al. 2012). Sensitivity of the technique depends on the spatial scale at which the survey is applied. Experimental research under controlled conditions in a fish breeding pond showed that, after inclusion of American bullfrog larvae in life-nets at a fixed position in the pond, the detection probability was significantly affected both by the distance from the life nets at which the water samples were taken, as well as by the density of the larvae in the life net (Brys et al. in prep). Interestingly, analyses of merged samples obtained from a large number of subsamples taken around the pond (every 5 m) revealed positive detection, even at the lowest density. Because eDNA can occur very clustered in lentic waters, due to its limited dispersion capacity and relative fast decay rates, spatial sampling and merging of subsamples is needed to obtain efficient detection probabilities, especially if the target species occurs at low densities or shows a limited action radius within the water.</td></tr>
<tr><td>Effort required
e.g. period of time over which measure needs to be applied to have results</td><td>Depending on its size, per pond generally 1 to 2 hours of fieldwork are needed to obtain a full-covering spatial sampling in order to appropriately test for the presence/absence of American bullfrog individuals via eDNA. Upon engaging in eDNA sampling programs, and based on work performed in Belgium, in case only adults are present, eDNA analyses should be carried out when they are most active in the water column and detection probabilities are consequently highest. When larvae are present, year round sampling can be applied, but again detection probabilities will be highest when they are most active. In addition, turbidity of the water can inhibit amplification success and thus detection efficiency. Water sampling is consequently preferred when the water is as clear as possible, and when bullfrog adults, larvae or both are most active. However, since bullfrog often inhabits turbid water, extra challenges are posed during the stage of eDNA extraction, purification and amplification in order to avoid PCR inhibition and the occurrence of false negatives.</td></tr>
<tr><td>Resources required [1]
e.g. cost, staff, equipment etc.</td><td>Sampling and analysis is best performed with two staff members. However, personnel cost can be reduced by sending the samples to dedicated labs. Below, a rough estimation is given on the costs associated with one eDNA analysis for an “average-sized” pond. It is clear that handling costs significantly depend on the size of the pond and the qualification of the scientists/technicians/laborants who are conducting the work, as well as the protocol available at the moment.</td></tr>
</table>

Equipment / staff	*Costs (€ or hours)*
*Sampling kit (cost)**	*20.5 €*
Lab consumables (cost)	*21.5 €*
Field sampling (hours)	*1.5 – 2.0*
Lab work (hours)	*0.35*
Total cost	*79 – 89 €*

** this includes the cost of an enclosed filter.*

If a larger number of ponds/samples need(s) to be investigated, additional costs for negative blanco's and positive controls (one or two per 20-25 samples) need to be taken into account.

Side effects (incl. potential) – both positive and negative
i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc.

For each of the side effect types please select one of the impact categories (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.

Environmental effects	*Positive*		*Neutral or mixed*	*X*	*Negative*	
Social effects	*Positive*		*Neutral or mixed*	*X*	*Negative*	
Economic effects	*Positive*		*Neutral or mixed*	*X*	*Negative*	

Rationale:
The method has very little side effects and is non-invasive. At most, slight disturbance of the banks can be caused, although sampling by using a long pole with a sampling bag at the end can easily overcome any disturbance to the environment. Sampling material, such as boots, pole, tubes, syringe etc. need to be sterilised when re-used between different ponds, to avoid cross-contamination with target DNA between different ponds, and to avoid spread of invasive and/or pest species such as New Zealand pygmyweed *Crassula helmsii*, Nuttall's pondweed *Elodea nuttallii,* Canadian waterweed *E. Canadensis,* curly waterweed *Lagarosiphon major,* water Fern *Azolla filiculoides,* flowering water primrose *Ludwigia grandiflora,* floating pennywort *Hydrocotyle ranunculoides* or parrot's feather *Myriophyllum aquaticum*. Also, to prevent further spread of amphibian disease such as *Ranavirus* or chytrid fungus *Bd* and *Bsal*, several hygiene protocols are available (check, clean, dry) (e.g. Froglife 2016, RAVON 2017).

Acceptability to stakeholders
e.g. impacted economic activities, animal welfare considerations, public perception, etc.

Please select one of the categories of acceptability (with an 'X'), and

Acceptability to stakeholders	*Acceptable*	*X*	*Neutral or mixed*		*Unacceptable*	

Rationale:
In comparison to traditional monitoring methods, eDNA approaches are less expensive and do not have any

provide a rationale, with supporting evidence and examples if possible.	negative impact on the aquatic environment, if applied appropriately.
Additional cost information [1] When not already included above, or in the species Risk Assessment. - implementation cost for Member States - the cost of inaction - the cost-effectiveness - the socio-economic aspects Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	No information available.
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Inconclusive* ☐ *Unresolved* ☐ *Established but incomplete* ☐ *Well established* *X* *Rationale*: The method of eDNA is already extensively tested under controlled and natural conditions, and can thus be assumed to be a well-developed method that is proven to be a very reliable and cost-effective for species detection, such as bullfrogs, in aquatic environments.

Rapid eradication for new introductions - Measures to achieve eradication <u>at an early stage of invasion</u>, after an early detection of a new occurrence (cf. Article 17). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory. **This table is repeated for each of the eradication measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Drainage of water bodies.** Successful eradications in Europe have only been achieved at an early stage of invasion (Theismeier et al. 1994; Banks et al. 2000; Ficetola et al. 2007). After early detection of a newly established population, eradication is possible using a **combination of repeated drainage with a suite of active trapping methods** (fyke netting, seine netting, electrofishing, hand capture, night lighting etc.). To prevent dispersal of larvae, additional filters should be

	placed at the intake and/or outflow points during drainage. Simultaneously, a fence should be placed at the fringe of the pond to prevent post-metamorphic dispersal during the actions. Drainage of the waterbody disrupts larval development, as American bullfrogs require a period of permanent water. The duration of this period depends on, amongst other factors like food competition and predation, the water temperature, and can range from 5 months in the southeastern United States and Mexico, up to 4 years at the northernmost extent of their range (Govindarajulu 2004; Pearl et al. 2004). In Belgium, the Netherlands and France, at least 2 years of permanent water are needed for the development of the larvae (Jooris 2005; Devisscher et al. 2012). Interrupting this period of continuous water need, will result in increased mortality among the larvae (D'Amore et al. 2009). To completely prevent larval development, yearly drawdown actions should be performed. Population models indicate that methods focusing on larval mortality are most effective when a complete removal of larvae is achieved (Govindarajulu 2004; Adams & Pearl 2007). Thus, the pond should be given the time to dry up completely. However, when complete dry up is not achievable, not desirable or just when animal welfare perception is important, a reduction of water depth already allows for more efficient captures with seine nets, provided the bottom of the ponds are free of obstructions (Devisscher et al. 2012). Devisscher et al. (2013) also describe complete habitat conversion of an infested pond to a type of grassland, which involved draining the pond and filling it up with substrate from the ditches around.
Scale of application At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	Complete drainage of water bodies is used around the world to rapidly respond to newly discovered populations, however mostly in combination with other methods (Devisscher et al. 2012). For example, in Flanders, complete drainage of a 1,000 m^2 waterbody (estimated volume 1,500 m^3) was performed after a period of intensive capturing of larvae with double fyke nets. During drainage, fencing was applied (Devisscher et al. 2013). Drainage was performed using a 40 m^3/h flow rate pump with an 8 mm filter installed on the suction head, assuming smaller tadpoles would not survive this operation. Often, complete drainage of a pond or waterbody can be difficult to achieve, especially in areas with upwelling groundwater. Hence, larvae remain in the remaining water and mud. Seine netting or electrofishing are poorly effective to collect remaining larvae in such situations. In the Netherlands, in 1990, it was confirmed that a bullfrog population successfully reproduced in a garden pond (250 m^2) in Breda since 1989 (Stumpel 1992). An eradication campaign was launched in 1990 and, by 1991-1992, this population had been wiped out (Veenvliet & Veenvliet 2002).

<table>
<tr><td>Effectiveness of the measure
Is it effective in relation to its objective? Has the measure previously worked, failed?

Please select one of the categories of effectiveness (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td><td>

Effectiveness of measures	*Effective*	*X*	*Neutral*		*Ineffective*		*Unknown*	

Rationale:
Examples of effective use of drainage, combined with active trapping methods, are numerous in Europe (see examples above). For instance, in 2010, a population was discovered in two breeding ponds in Baarlo (Limburg, Netherlands). After a thorough inventory, the populations in the two ponds were effectively removed through a combination of fencing, seine netting, fyke netting, electrofishing and drainage of one pond (Creemers 2011a,b). Nearby sites were screened for bullfrog presence with eDNA techniques (Crombaghs 2012; Goverse et al. 2012). In Lower Saxony (Germany), a population was successfully eradicated by drainage (Veenvliet & Veenvliet 2002). In 2002, a population in Meckenheim near Bonn (Germany) was partially eradicated, involving fencing and several drainage actions (Laufer & Waitzmann 2002). In south east England (UK), populations in a series of (fish) ponds in a stream valley surrounded by mixed farmland and woodland were 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) and the population is believed eradicated (GB Non-Native Species Secretariat).</td></tr>
<tr><td>Effort required
e.g. period of time over which measure needs to be applied to have results</td><td>Population models suggest that, given an isolated pond, 10 years of annual drawdowns (resulting in 100% tadpole mortality) are required to eradicate a local bullfrog population (Doubledee et al. 2003; Maret et al. 2006). Bi-annual drawdowns combined with a 55%, 65% and 75% increase in adult mortality will extend the period needed for eradication to 40, 25 or 15 years, respectively (Doubledee et al. 2003; Maret et al. 2006). Less frequent drawdowns or immigration into the pond will result in more delayed and/or lack of eradication success.</td></tr>
<tr><td>Resources required [1]
e.g. cost, staff, equipment etc.</td><td>Highly dependent on the situation. If (manual) outlets are present, the only costs would be placing of fences and filters to prevent dispersal. Additionally, some effort can be done to reduce non-target damage by removing wanted species prior to complete dry up. Also, seine netting can be performed when animal welfare perception is important or when complete dry up is not achievable. When no outlets are present, the costs for pumping equipment and/or installation of outlets should be included. Additional costs could include the replacement of permanent inlets with more controllable variants.
A German management campaign which pumped out five infested ponds twice to remove adults and tadpoles, supplemented with electrofishing, with the help of 20 volunteers and the local fire department, documented the following costs (Weizmann 2002): 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</td></tr>
</table>

<table>
<tr><td></td><td>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) (Reinhardt et al. 2003; EEA 2012). In the UK (south east England), a population was eradicated in a series of (fish) ponds in a valley (Langton et al. 2011). The vicinity of the original breeding sites (two fish ponds) was subjected to detailed monitoring and the populations were 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 cost of around 100,000 £ across seven ponds (EEA 2012).</td></tr>
<tr><td>Side effects (incl. potential) – both positive and negative
i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc.

For each of the side effect types please select one of the impact categories (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td><td><table>
<tr><td>Environmental effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td></td><td>Negative</td><td>X</td></tr>
<tr><td>Social effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
<tr><td>Economic effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
</table>
Rationale:
Any change in the hydroperiod of an aquatic ecosystem has the potential of creating negative non-target effects. Some of the negative effects can be prevented by capturing and translocating individuals of native/desired species prior to complete drawdown. Still, some mortality amongst these species is to be expected during such operations. Non-target impact on native amphibians can be mitigated by adapting the period in the year to perform drawdown of a pond (Maret et al. 2006). For example, in northwest Europe, most native species of frog, toad and newt would be impacted by drainage in the period March-July (Adams & Pearl 2007; Werner et al. 2007). Some non-target species can profit from these temporary dry periods, for example because of reduced predation pressure (e.g. some dragonfly species).</td></tr>
<tr><td>Acceptability to stakeholders
e.g. impacted economic activities, animal welfare considerations, public perception, etc.

Please select one of the categories of acceptability (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td><td><table>
<tr><td>Acceptability to stakeholders</td><td>Acceptable</td><td></td><td>Neutral or mixed</td><td>X</td><td>Unacceptable</td><td></td></tr>
</table>
Rationale:
Draining ponds can invoke negative reactions from the public, conservationists and conservation managers who might worry about the negative effects on non-target native species. Also, if ponds are used for angling, anglers might oppose.</td></tr>
<tr><td>Additional cost information [1]</td><td>No information available.</td></tr>
</table>

When not already included above, or in the species Risk Assessment. - implementation cost for Member States - the cost of inaction - the cost-effectiveness - the socio-economic aspects Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Inconclusive* ☐ *Unresolved* ☐ *Established but incomplete* ☐ *Well established* *X* *Rationale*: Although some articles only report partial eradication (e.g. Laufer & Waitzmann 2002), there are multiple examples in the literature of successful eradication actions on newly discovered populations, some of which even appeared to have been present for a longer time, at various scales (individual garden or fish ponds, but also invaded areas with several ponds where bullfrog reproduction). This has been done using a combination of drainage with a suite of active trapping methods (mostly electrofishing and hand captures).

Rapid eradication for new introductions - Measures to achieve eradication <u>at an early stage of invasion</u>, after an early detection of a new occurrence (cf. Article 17). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory. **This table is repeated for each of the eradication measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Fyke netting.** Fyke netting is a form of active trapping that is mostly useful to reduce, or all together remove (at early stages of invasion), the larval segment of a bullfrog population. Adult bullfrogs can also be caught using fyke nets (Devisscher et al. 2012). Although population models indicate removal of larvae to be rather inefficient (Govindarajulu et al. 2005), fyke netting is practical, equipment is relatively cheap and the large amounts of larvae caught with some

	accidental post-metamorphic individuals can render this method efficient for small, shallow ponds (Devisscher et al. 2012; Louette et al. 2012a,b). Based on extensive trials in Belgium, fyke nets have a consistent capture success rate of about 6% of the larval population (Louette et al. 2012b) and about 0.7% (0.3%-1.2%) for post-metamorphic stages per unit of effort (i.e. 1 fyke net harvested in 24h) (Devisscher et al. 2013). Research showed that double fyke nets are highly effective and efficient for capturing bullfrog larvae. Simulations show that the simultaneous use of eight double fyke nets in a pond, at twelve separate catch occasions, can bring larval numbers below a critical threshold. At the same catch intensity and effort, 46% of the adult population is equally removed (or 4 out of 9 bullfrog adults present on a stretch of 200 m shoreline). Thus, nearly half of the adult segment will no longer take part in reproduction. Consequently, when the captures are continued over several years and combined with other methods to prevent successful reproduction (egg removal, fencing of ponds), this may lead to the control or eradication of isolated populations. For a more realistic assessment of the effect of the simultaneous removal of the adult segment using double fyke nets, the application of dynamic population models is a possibility. In a Belgian trial, the catchability of adults, using one double fyke net for 24 h, was rather stable and equalled 0.7% of the population size. This can provide a better understanding of the way that current management impacts on the demography of bullfrog populations and the probability of achieving the management objectives (eradication, control). eDNA approaches can offer an appropriate tool to evaluate the success of eradication programmes. To ascertain complete eradication, an eDNA sampling campaign can offer additional insights on whether there are still surviving bullfrog individuals at previously infested locations or not, and thus for confirmation of eradication success (Ficetola et al. 2008b; Adriaens et al. 2013). Experimental work indeed revealed that when bullfrog individuals are artificially introduced in a pond for some weeks by using life-nets, and subsequently removed again, the species can no longer be detected after one week (Brys et al. in prep). A yearly monitoring campaign could be implemented starting from the second year of the campaign until 5 years after the end of the campaign to ensure complete eradication (van Delft et al. 2013).
Scale of application At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	Effective eradication of an early stage invasion with fykes has been performed in a pond complex in the Campine area of Belgium at Kasterlee (INBO, unpublished data). The complex had an overall scale of approximately 15 ha and consisted of nine ponds with an average size of 1100 m^2 each. Eradication commenced in 2014 and, since 2017, the complex is considered bullfrog free.

Effectiveness of the measure Is it effective in relation to its objective? Has the measure previously worked, failed? Please select one of the categories of effectiveness (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Effectiveness of measures***</td><td>*Effective*</td><td>*X*</td><td>*Neutral*</td><td></td><td>*Ineffective*</td><td></td><td>*Unknown*</td><td></td></tr></table> *Rationale*: When the full extent of the invasion is known, and limited to easily accessible ponds with a maximum size of 2000 m^2, the method has proven to be very effective in eradicating the species. However, when a larger and/or less accessible body of water is invaded, efficiency is greatly reduced (Devisscher et al. 2013). Based on a population model (Doubledee et al. 2003), Grey (2009) concluded that removing about 70% of the larvae from permanent ponds through fyke netting (or other methods) every two years should be enough to keep a bullfrog population in check. Additionally, the method has been successfully used in combination with draining and removal of water bodies at Hoogstraten in the north of Belgium.
Effort required e.g. period of time over which measure needs to be applied to have results	The effort required depends on the local situation and the stage of invasion upon bullfrog detection. Overall, 5 years of intensive fyke netting (plus 5 years post-treatment) are sufficient to deplete a population. During the period of intensive fyke netting, a minimum of four actions (+- 5 fykes per 24h) should be performed in every pond yearly, in a 750 m range around the first sighting. Depending on population densities (to be determined with one capture per unit of effort), this intensity can increase to 20+ actions (Devisscher et al. 2013). Depending on pond size and population density, between 2 and 10 fykes (5 per pond is most cost-efficient) should be placed in the pond until low levels of larvae (<10 per capture) are harvested per 24h (Louette 2012). Subsequent monthly control captures should be conducted for 5 subsequent years to make sure no revival of the larval population is missed, scaling up again when more larvae are caught. Post-treatment can be performed by eDNA sampling or fyke netting at four actions per year. A trained team can harvest around 10 fykes per day (8h per day including transportation of people and equipment). Placing and harvesting of fykes is best performed by two staff members. The estimated effort needed to eradicate or control a local population can be calculated based on the capture size of a single trial capture event. Such trial capture should consist of at least 4 fykes placed for 24 hours in each pond. The amount of larvae caught is then divided by the actual amount of fykes used and multiplied by 26 to estimate the size of the initial population. Alternatively, the formula "1 – (1 – 0.06) ^ number of double fyke nets" can be used to calculate the removal rate. Plotting the removal rate starting from the initial population size will enable derivation of the number of actions needed (Louette et al. 2012a; Devisscher et al. 2013). For instance, to reduce a starting population of 5,000 larvae to less than 10 individuals, about 20 CPUE are needed. On average, a team of three people needs 45-60 minutes to harvest one fyke net (incl. registration of numbers needed). To install a fyke, one should count on 30 minutes.

<table>
<tr>
<td>Resources required [1]
e.g. cost, staff, equipment etc.</td>
<td>Louette et al. (2012a) estimated the cost for one management season to reduce the larval segment of a bullfrog population to below 10 and 100 individuals, using 2, 5 and 8 fykes per pond, and starting from a low (1,000 larvae) and high (5,000) abundance of larvae. The total cost, as estimated per pond, per season, can be as low as 1,848€ (13 actions with 5 fykes to achieve a 100 larvae goal starting at low abundance) and increase to 6,210€ (45 actions with 2 fykes to achieve a 10 larvae goal starting at high abundance). Prices include labour costs, material depreciation and transportation. The price per unit of effort, using 2, 5 or 8 fykes per pond, will be around 138€, 308€ and 478€, respectively. The difference in price per unit of effort is caused by a reduction of the cost of transportation. This causes the price per unit of effort to increase in a nonlinear way, flattening off until the maximum number of fykes harvestable per day is reached (about 10 fykes for a team of two). For the equipment, one double fyke net costs between 700€ and 900€.</td>
</tr>
<tr>
<td>Side effects (incl. potential) – both positive and negative
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For each of the side effect types please select one of the impact categories (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td>
<td>
<table>
<tr><td>Environmental effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
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<tr><td>Economic effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
</table>

Rationale:
The use of double fyke nets allows for replacement of bycatch (fish or other non-target species) 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, MS222 (tricaine methanesulfonate) or clove oil is considered humane, fast, efficient and harmless for the environment (Close et al. 1997). By preference, and for reasons of user-friendliness, humane killing is best performed in a separate closed container, thereby avoiding any chemical release into the environment. The method has no documented social or economic impacts.
</td>
</tr>
<tr>
<td>Acceptability to stakeholders
e.g. impacted economic activities, animal welfare considerations, public perception, etc.

Please select one of the categories of acceptability (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td>
<td>
<table>
<tr><td>Acceptability to stakeholders</td><td>Acceptable</td><td>X</td><td>Neutral or mixed</td><td></td><td>Unacceptable</td><td></td></tr>
</table>

Rationale:
As bycatch (fish or other non-target species) can be replaced in good condition, the method is mostly acceptable to stakeholders (e.g. anglers, pond owners). It should be noted that fyke nets are known to be regularly vandalised, although if information (name, photo, organisation, telephone number, purpose) is attached to the capture gear, it can largely prevent this from happening.
</td>
</tr>
</table>

Additional cost information [1] When not already included above, or in the species Risk Assessment. - implementation cost for Member States - the cost of inaction - the cost-effectiveness - the socio-economic aspects Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	In Belgium, 10,890 € were paid in a year (2017-2018) by the Agency for Nature and Forest to a company for fyke netting in a complex of ponds (Antwerp province, Arendonk). In total, this represented 81 catch per unit efforts (1 fyke per 24h) on 12 ponds (total surface area 0.8 ha) in a total project area of 35,000 m². The only known population in Wallonia (0.3 ha surface area) is actively managed with fyke nets (30 weeks or 60 hours per year) since 2016, at a cost of 16,000 € including salary of one person to empty the fykes (number of traps ranging from 22-42) and equipment (pers. comm. E. Branquart).
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Inconclusive* | | *Unresolved* | | *Established but incomplete* | X | *Well established* | *Rationale*: Effectiveness of fyke netting on bullfrog larval population has been studied. However, gaps exist with regards to the effectiveness of this method to also remove the juvenile and adult segments of bullfrog populations.

Rapid eradication for new introductions - Measures to achieve eradication <u>at an early stage of invasion</u>, after an early detection of a new occurrence (cf. Article 17). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory. **This table is repeated for each of the eradication measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Use of chemicals.** The application of chemicals, while used with varying levels of success around the world, is prohibited for use in aquatic environments in the European Union (Council Directive 76/464/EEC). However, chemicals can effectively be used elsewhere to eradicate American bullfrogs, either by application of biocides to the freshwater environment or by spraying them on individual frogs. Witmer et al. (2015) tested rotenone, amongst other chemicals. Rotenone, which has been widely used to eradicate non-native fish (and also Union List crayfish) populations, has proven to be

<table>
<tr><td></td><td>lethal to bullfrog at a 1% solution, when combined with a 4.6% solution of permethrin. Draining followed by liming, a method used to disinfect aquaculture facilities, has been proposed for crayfish management and is potentially also suited for American bullfrog (Basilico et al. 2013). Alternatively, a 5% chloroxylenol or a 10% caffeine solution can be used. A reduction of the water level prior to application is advised to increase efficacy (Witmer et al. 2015). The use of chemicals can, however, create a habitat free of predatory fish, which is ideal for amphibians like bullfrogs, thus allowing resettlement at increased speed (Watson 2005). To avoid this, great care should go into preventing recolonisation by fencing and placing filters, if applicable.</td></tr>
<tr><td>Scale of application
At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.</td><td>The application of chemicals to kill American bullfrog has only been tested in laboratory conditions.</td></tr>
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Is it effective in relation to its objective? Has the measure previously worked, failed?

Please select one of the categories of effectiveness (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td><td><table><tr><td>Effectiveness of measures</td><td>Effective</td><td></td><td>Neutral</td><td></td><td>Ineffective</td><td></td><td>Unknown</td><td>X</td></tr></table>
Rationale:
The application of chemicals to kill American bullfrog has only been tested in laboratory conditions.</td></tr>
<tr><td>Effort required
e.g. period of time over which measure needs to be applied to have results</td><td>The application of chemicals to kill American bullfrog has only been tested in laboratory conditions.</td></tr>
<tr><td>Resources required [1]
e.g. cost, staff, equipment etc.</td><td>Not yet applied.</td></tr>
<tr><td>Side effects (incl. potential) – both positive and negative
i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc.</td><td><table><tr><td>Environmental effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td></td><td>Negative</td><td>X</td></tr><tr><td>Social effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr><tr><td>Economic effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr></table>
Rationale:</td></tr>
</table>

<table>
<tr><td>For each of the side effect types please select one of the impact categories (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.</td><td>The biocides used are not specific and can cause severe non-target mortality (Snow & Witmer 2010). Their use should be limited to cases where almost no non-target animals are present or where the impact of American bullfrog settlement is greater than the expected impact of using chemicals. Often drawdown is needed for the application of biocides to be highly effective.</td></tr>
<tr><td>Acceptability to stakeholders
e.g. impacted economic activities, animal welfare considerations, public perception, etc.

Please select one of the categories of acceptability (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.</td><td><table><tr><td>Acceptability to stakeholders</td><td>Acceptable</td><td></td><td>Neutral or mixed</td><td></td><td>Unacceptable</td><td>X</td></tr></table>
Rationale:
Classically, the use of toxic substances, chemicals and biocides invokes resistance from the public, especially if they are used around water. Spraying caffeine on bullfrog skin is not a humane method for killing amphibians (Close et al. 1997; European Commission 1997) and may face negative reactions from the public based on animal welfare considerations.</td></tr>
<tr><td>Additional cost information [1]
When not already included above, or in the species Risk Assessment.
- implementation cost for Member States
- the cost of inaction
- the cost-effectiveness
- the socio-economic aspects

Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).</td><td>No information available.</td></tr>
<tr><td>Level of confidence on the information provided [2]

Please select one of the confidence categories along with a statement to support the category chosen. See Notes section at the bottom of this document.
NOTE – this is not related to the</td><td><table><tr><td>Inconclusive</td><td></td><td>Unresolved</td><td></td><td>Established but incomplete</td><td>X</td><td>Well established</td><td></td></tr></table>
Rationale:
Although studies have been performed looking at direct mortality of various chemical compounds as suppression agents for bullfrogs (e.g. Finlayson et al. 2000; Rayner & Creese 2006; Abbey-Lambertz et al. 2014), the literature only describes laboratory tests or direct skin application, instead of real field studies. Also, the effect on non-target</td></tr>
</table>

effectiveness of the measure	species is not well studied.

Management - Measures to achieve management of the species once it has become widely spread within a Member State, or part of a Member State's territory. (cf. Article 19), i.e. **not** at an early stage of invasion (see Rapid eradication table above). These measures can be aimed at eradication, population control or containment of a population of the species. **This table is repeated for each of the management measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Changing the hydroperiod.** Bullfrog larvae can take up to two years to metamorphose. During this period, they need permanent water (Devisscher et al. 2012). Hence, adaptation of hydrology or temporary draining can be used to control the species. Similarly to the drainage of water bodies, changing the hydroperiod can disrupt larval development by interrupting the period of permanent water needed for development (D'Amore et al. 2009). Changes to the hydroperiod are a long term method, compared to draining or permanent pond removal or destruction. To effectively impact on larval development, altering the hydroperiod can only be effective if the pond is left dry for a sufficient period of time each year. If this is repeated several years, this could potentially result in effective control. Adjustments of the hydrology include one-off interventions that can ensure the waterbody dries out more frequently and for longer periods of time. The hydroperiod can be changed by altering the depth of a waterbody or by altering the intake of water (Devisscher et al. 2012; Devisscher et al. 2017). Depending on the local duration of larval development, in the case of a two year development cycle, an annual drought of at least a week is needed (Devisscher et al. 2017). Examples of such interventions include short-term drainage, the partial filling of ponds to encourage temporary dry out in summer, the reprofiling of the pond floor or the closure of inlets. This method has also been proposed for other non-native species, such as pumpkinseed *Lepomis gibbosus* (van Delft et al. 2013). Creation of a cycle of periodical droughts might discourage reproduction in the waterbody affected. However, this in turn might result in a shift of focus of the reproduction effort to other nearby water bodies, while still functioning as refuge for juveniles (Jooris 2005; Devisscher et al. 2017). A combined approach in a wide area is thus required. This can be achieved by reducing the suitable habitat for reproduction and focusing larval removal effort in the remaining suitable water bodies, while increasing the overall post-metamorph mortality and limiting their dispersal. 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 (D'Amore et al. 2009). 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 adjustments refer to Devisscher et al. (2012).
Scale of application At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	No documented applications of the method for bullfrog have been described, but will probably become available during the course of the LIFE project *Control strategies of Alien Invasive Amphibians* (www.life-croaa.eu), which aims to tackle the French bullfrog population using a variety of methods and strategies.
Effectiveness of the measure Is it effective in relation to its objective? Has the measure previously worked, failed? Please select one of the categories of effectiveness (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>Effectiveness of measures</td><td>Effective</td><td>X</td><td>Neutral</td><td></td><td>Ineffective</td><td></td><td>Unknown</td><td></td></tr></table> *Rationale*: If water levels on a site are under human control, drying down sites while keeping in mind the needs of native species can be an excellent means of removing the possibility of bullfrog breeding, at the same time fostering the continued presence of native amphibian species (D'Amore et al. 2009). Also, native species will profit from the removal of exotic fish, and drying can be very effective to this end (Banks et al. 2000; Bringsoe et al. 2002; Foster & Banks 2008; Snow & Witmer 2010). Maret et al. (2006) provide information on this method.
Effort required e.g. period of time over which measure needs to be applied to have results	The method should be implemented for at least 5 years after the last American bullfrog population in the wider environment is eradicated (based on Maret et al. 2006).
Resources required [1] e.g. cost, staff, equipment etc.	Implementing a change of the hydroperiod will have a large initial cost, but very low, or no, post-treatment costs. Possible costs for the method will depend on several factors, such as the measure adopted (reducing pond depth or alteration of water intake(s)), size and depth of the waterbody, number of intakes, groundwater level, etc. Indicative prices cannot be given, as there are no documented applications of the method.
Side effects (incl. potential) –	

both positive and negative i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc. For each of the side effect types please select one of the impact categories (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Environmental effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td></td><td>*Negative*</td><td>*X*</td></tr><tr><td>***Social effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td>*X*</td><td>*Negative*</td><td></td></tr><tr><td>***Economic effects***</td><td>*Positive*</td><td></td><td>*Neutral or mixed*</td><td>*X*</td><td>*Negative*</td><td></td></tr></table> *Rationale*: Like drawdown, changing the hydroperiod is suspected to have negative effects on most aquatic non-target species, while having a possible positive effect on others. The passive nature of this method might result in increased mortality amongst non-target species, mostly fish, but also, depending on the timing of application, native amphibians, invertebrates, macrophytes etc. Other biota, such as some dragonfly species, might on the contrary profit from a temporary situation where predation pressure in the waterbody is kept at very low levels. To reduce non-target effects, active removal and translocation of non-target species prior to the induced changes can be performed. This method has no known positive or negative social or economic effects.
Acceptability to stakeholders e.g. impacted economic activities, animal welfare considerations, public perception, etc. Please select one of the categories of acceptability (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Acceptability to stakeholders***</td><td>*Acceptable*</td><td></td><td>*Neutral or mixed*</td><td>*X*</td><td>*Unacceptable*</td><td></td></tr></table> *Rationale*: Documented cases are not available, but presumably the initial high cost and the potential non-target effects on native biota might invoke some problems with stakeholders.
Additional cost information [1] When not already included above, or in the species Risk Assessment. - implementation cost for Member States - the cost of inaction - the cost-effectiveness - the socio-economic aspects Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	No information available.
Level of confidence on the information provided [2]	<table><tr><td>*Inconclusive*</td><td>*X*</td><td>*Unresolved*</td><td></td><td>*Established but*</td><td></td><td>*Well established*</td><td></td></tr></table>

Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	incomplete *Rationale*: No documented applications of the method for bullfrog were found.

Management - Measures to achieve management of the species once it has become widely spread within a Member State, or part of a Member State's territory. (cf. Article 19), i.e. **not** at an early stage of invasion (see Rapid eradication table above). These measures can be aimed at eradication, population control or containment of a population of the species. **This table is repeated for each of the management measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Active trapping.** Active trapping of adult and metamorph stages using a suite of trapping methods can be a valuable part of an integrated pest management strategy for American bullfrog (active trapping of larval stages is discussed in rapid eradication > fyke netting). Removing part of the bullfrog population can help alleviate depredation, interspecific competition and disease transmission for native species (Snow & Witmer 2011). Potential methods for active trapping include hand capture (supported by nightlighting), netting, electrofishing, trapping (e.g. multicapture traps, carpet refuges placed on land, bottle traps in ponds), spearing (gigging), and shooting (discussed separately). Bullfrogs, being active both at night and throughout the day, are very shy, which hampers unaided active trapping. However a strong spotlight can be used to stun the bullfrogs, enabling easy capture (D'Amore et al. 2009; Orchard 2011). Active trapping is best performed in the period between late spring and early fall, when American bullfrogs start hibernating. Bullfrogs hibernate at the bottom of the pond or underground/leaves in nearby alluvial forests (Devisscher et al. 2012). To extend this active trapping season to autumn and winter, sniffer dogs can be used to find hibernating bullfrogs. These bullfrogs will most likely be sluggish, and thus easier to capture. Snow and Witmer (2011) tested a multicapture trap designed for cane toad (FrogWatch, Darwin, Australia), using fishing lures, live crickets and lights as attractants. They concluded that fishing lures were most attractive to bullfrogs. Descamps and De Vocht (2012) tested alternative lures (e.g. banana, pork meat, liver, peach) under controlled trials in the lab and concluded that lures with high blood content were more attractive to larvae. Whether this also holds for adults is unknown. If trapping is performed, it is advisable to use floating traps, as placing the traps on the shore may not be as effective. Bullfrogs use floating devices as roost platforms and flotation devices make the entire pond accessible to trapping, not just the shoreline (Snow & Witmer 2011). An adapted design was tested in Flanders during Invexo

	Interreg, but was unsuccessful in trapping subadults and adults (Devisscher et al. 2012). Berroneau (2007) also developed and used a trap (abri-flottant) based on this principle, which has since been used extensively in France with some success (Moissonier et al. 2007).
Scale of application At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	Snow and Witmer (2011) found some evidence to suggest that removing bullfrogs using a basic integrated pest management strategy (IPM) may have reduced the abundance of bullfrogs in small, heavily infested ponds in Colorado (0.002 km^2).
Effectiveness of the measure Is it effective in relation to its objective? Has the measure previously worked, failed? Please select one of the categories of effectiveness (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	<table><tr><td>***Effectiveness of measures***</td><td>*Effective*</td><td></td><td>*Neutral*</td><td>*X*</td><td>*Ineffective*</td><td></td><td>*Unknown*</td><td></td></tr></table> *Rationale*: Management strategies solely based on active trapping have limited ability to eradicate bullfrogs, because bullfrog populations exhibit strong density dependence and experience shows that reducing densities results in increased reproduction and survival, if not all bullfrogs are removed (Werner et al. 1995; Altwegg 2002; Doubledee et al. 2003; Govindarajulu 2004). However, active trapping can be part of an effective, integrated control strategy (e.g. Snow & Witmer 2011). Removing adults from the population also releases metamorphs from cannibalistic predation pressure (Govindarajulu 2004). For comparison, for cane toads it has been predicted that high intensity trapping, removing 25-40% of the population, is needed to achieve effective control (McCullum 2006). Active trapping methods are also labour and time intensive and do not reduce bullfrog numbers to the desired level. The level of success of active trapping varies greatly between sites, for example due to differences in bullfrog densities, weather conditions (cold nights) or low activity level of bullfrogs, e.g. at the end of summer when availability of insect prey is low (Snow & Witmer 2011). Lastly, as has been illustrated with cane toad management (Schwarzkopf & Alford 2002, 2007), males are more active wanderers and have bigger home ranges, which renders them more prone to end up in traps, whereas removing females is likely more critical for effectively reducing a population (Bury & Whelan 1984).
Effort required e.g. period of time over which measure needs to be applied to have results	The method is labour intensive and the effort required would be considerable. For example, Snow and Witmer (2011) cumulatively caught 18 bullfrogs in 10 trap nights, but this hardly represented 10% of the numbers present during peak season, as determined by audio counts.
Resources required [1]	As active trapping is possible through a number of different methods and, in practice, is mostly integrated with

<table>
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<td>e.g. cost, staff, equipment etc.</td>
<td>other methods, it is difficult to provide information on costs. It can be assumed that human resources (trapping hours) would represent a relatively bigger cost than many of the trapping materials e.g. a commercially available cane toad trap would be around 40€ (www.gettrapped.com).</td>
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<tr>
<td>Side effects (incl. potential) – both positive and negative
i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc.

For each of the side effect types please select one of the impact categories (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td>
<td>
<table>
<tr><td>Environmental effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
<tr><td>Social effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
<tr><td>Economic effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
</table>

Rationale:
Using nets and traps humanely requires regular (daily) checking, to release bycatch of native amphibians or other species alive and in good condition. In their multicapture trial, Snow and Witmer (2011) captured a single non-target frog on a total of 20 trap nights and note that using appropriate mesh sizes allowing smaller animals to escape the trap can reduce the amount of non-targets caught. In general, a good trap design limits non-target bycatch. No social or economic effects of placing traps in ponds are documented.</td>
</tr>
<tr>
<td>Acceptability to stakeholders
e.g. impacted economic activities, animal welfare considerations, public perception, etc.

Please select one of the categories of acceptability (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td>
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<table>
<tr><td>Acceptability to stakeholders</td><td>Acceptable</td><td>X</td><td>Neutral or mixed</td><td></td><td>Unacceptable</td><td></td></tr>
</table>

Rationale:
As bycatch is released in good condition and traps must be checked daily, animal welfare objections are probably not so much of an issue. In Belgium, it has been noted that people object to the presence of ugly, floating devices, such as big obvious traps e.g. in protected areas such as nature reserves, but this can be mitigated by camouflaging traps or raising awareness with the public. Vandalism can be an issue, but can be prevented by providing good on-site (information panels) and on-device (e.g. on traps and wildlife cameras) information about the control programme.</td>
</tr>
<tr>
<td>Additional cost information [1]
When not already included above, or in the species Risk Assessment.
- implementation cost for Member States
- the cost of inaction
- the cost-effectiveness</td>
<td>No information available.</td>
</tr>
</table>

- the socio-economic aspects Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	<table><tr><td>*Inconclusive*</td><td></td><td>*Unresolved*</td><td></td><td>*Established but incomplete*</td><td>*X*</td><td>*Well established*</td><td></td></tr></table> *Rationale*: The evidence for density dependence in established bullfrog populations is rather good, therefore management strategies solely based on trapping are indeed probably ineffective. However, little information is available on integrated pest management strategies that have actually investigated the relative contribution of active trapping of adult and metamorphs in reducing populations, as compared to other methods. Also, most studies that considered some forms of trapping alone do state that more information is needed on effective lures, efficiency (cost-benefit) and non-target effects (e.g. many non-target animals captured could be eaten in the trap before they are picked up).

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Measure description Provide a description of the measure, and identify its objective	**Shooting juvenile and adult bullfrogs.** This method involves culling the metamorph or adult stage with (air) rifles (equipped with a scope). Shooting allows managing American bullfrogs from a distance and provides benefits because the frogs are usually shy (D'Amore et al. 2009). The objective of shooting is to reduce the number of juvenile and adult American bullfrogs in a pond or population. The method is currently being tested in French and Belgian bullfrog populations (Michelin 2012; Sarat et al. 2016; unpublished data). Shooting should be performed preferably with a team of two people. One person typically locates the animals using a strong flashlight, binoculars or telescope, while the other person handles the rifle. To reduce the risk of ricochet when shooting around water bodies, it is recommended to use appropriate bullets that disintegrate immediately as soon as they hit anything. For example, Varmint bullets are equipped with a polymer (hard plastic) tip which is immediately shattered into small particles upon hitting an object. The following calibres of firearms seem suitable

	for shooting bullfrogs: .222 REM (Remington) preferably with Varmint tip V-Max 40 grains, .223 REM preferably with Varmint tip V-Max 40 or 50 grains, or .22-250 WIN (Winchester) preferably with Varmint tip V-Max 40 to 55 grains. Unlike firearms, air- and gas-powered weapons are much quieter and give less cause for disturbance. Ricochet is also more limited with these weapons, and often legislation allows a fixed silencer. The risk of ricochet can be reduced by using the right choice of ammunition (pellet) and by shooting from an elevated position. Current air- and gas-pressure weapons perform with enough precision to allow, provided a solid rifle scope is used, a target the size of a metamorph (juvenile) American bullfrog. However, this does require the necessary expertise and experience of the shooter and is certainly not available to everyone. The distance from where such a target can be hit is unclear, but could be examined by means of field tests. In addition, the industry has developed pellets with ballistic tips that improve their accuracy. The following calibres meet most minimum legal requirements and are technically suitable for eliminating bullfrogs with air and gas pressure weapons: .177 caliber (4.50 mm) preferably with 7 and 8 grains pellets, .22 caliber (5.58 mm) preferably with 14 to 16 grains pellets and .25 calibre (6.35 mm) preferably with 20 to 22 grains pellets. These guidelines are in no way generically applicable to all EU Member States and any application of shooting should be informed by the specific Member State weapons legislation. Because male American bullfrogs make noise, they are easier to detect than females or juveniles. Culling can be performed throughout the day, however actions at night are perceived to be more effective. Working at night increases the efficiency of shooting, because during the day animals are harder to find, especially at low densities. A disadvantage of night shooting is that American bullfrogs are harder to distinguish from native species, such as other green frogs (e.g. the common marsh frog *Pelophylax ridibundus*). Often, due to the limited visibility, there is not much more than a reflection of the eyes. Searching for bullfrogs can be supported by using sound. However, mostly only males will answer such call (Devisscher et al. 2012). Another potentially interesting tool to increase the probability of detection is the use of artificial islands (floating platforms) in the pond.
Scale of application At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	Sarat et al. (2016) report practical tests on shooting American bullfrog in Aquitaine (France) in pools of 1200 m² (6 sessions in the period 2004-2006), with the support of the Office National de la Chasse et de la Faune Sauvage. Here, shooting was carried out in shifts of two, with one person locating frogs with sound and light and the other using a .22 long rifle or an air rifle. During the control programme, 68 shooting sessions were held at 32 locations, with additional efforts of volunteers (Michelin 2012). On this basis, the authors recommend the use of an air rifle.

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<tr><td>Effectiveness of measures</td><td>Effective</td><td>X</td><td>Neutral</td><td></td><td>Ineffective</td><td></td><td>Unknown</td><td></td></tr>
</table>

Rationale:
As population models have indicated that the removal of juveniles from the population is more efficient to deplete a bullfrog population (Govindarajulu 2004; Govindarajulu et al. 2005), reducing juveniles and adults has a greater impact on the population than catching larvae, but is often more difficult to implement (D'Amore et al. 2009; Louette et al. 2014). Post-metamorphic stages are harder to detect and generally more shy, which hampers their effective control. Culling with (air) rifles is potentially very useful, as it allows management from greater distances (D’Amore et al. 2009). In France, shooting was used as a supplemental method to drainage and netting, and effectively reduced the spread of American bullfrog (Michelin 2012; Sarat et al. 2016). Feasibility tests in the Campine area in Flanders (Belgium) indicate air rifles with silencers to be very effective, as these cause hardly any disturbance, which allows for the shooting of several individuals in succession (INBO, unpublished data). The long term effectiveness of the method, in combination with fyke netting or other methods, as used in Belgium, has yet to be confirmed, e.g. through population modelling.
</td>
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<td>Effort required
e.g. period of time over which measure needs to be applied to have results</td>
<td>Eradication efforts in the Sologne region in France suggest that a sustained culling effort, combined with drawdown, seine netting and egg destruction, effectively reduced the density of the post-metamorphic stages in an American bullfrog population. Over a period of seven years, the presence of American bullfrogs was reduced from 83 ponds to only 29 (Michelin 2012; Sarat et al. 2016).</td>
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<tr>
<td>Resources required [1]
e.g. cost, staff, equipment etc.</td>
<td>The cost of application depends on the choices of equipment (type of rifle) and the number of staff or volunteers deployed. The method is best performed by at least two people, to locate and identify bullfrogs and to shoot them. Depending on the type of rifle, the cost can be less than €1000, up to more than €2000, excluding costs like a decent scope (ranging from €200 to more than €750), firing support (starts at €100) and some extras like binoculars (around €1000), a telescope (up to €2000) or a rangefinder (> €200). The prices of ammunition also vary greatly, from €1 to 100€. However, the cost of acquisition of a rifle and any extras are a one off investments and only the cost of the ammunition and staff are recurrent.</td>
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<tr><td>species, etc.

For each of the side effect types please select one of the impact categories (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.</td><td>Rationale:
The largest cause of non-target effects for this method is the probability of non-target amphibians getting shot, as misidentification is possible. However, in most European countries where bullfrog is established, this mostly concerns the native marsh frog P. ridibundus which, in general, is not threatened, is common or is even non-native in certain areas (Holsbeek et al. 2008, 2009, 2010; Holsbeek & Jooris 2010). Training to recognise the differences between these species, even in the dark, will reduce the chance of misidentification. The noise production associated with the use of firearms can cause disturbance to people or other wildlife on sites.</td></tr>
<tr><td>Acceptability to stakeholders
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Please select one of the categories of acceptability (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.</td><td><table><tr><td>Acceptability to stakeholders</td><td>Acceptable</td><td></td><td>Neutral or mixed</td><td>X</td><td>Unacceptable</td><td></td></tr></table>
Rationale:
There might be resistance against shooting in the field, because of owners being reluctant to allow rifles and firearms on their property (e.g. in nature reserves). For animal welfare reasons, the precondition for the use of weapons to eliminate a species is that the weapons used are effective at killing the animal quickly and accurately. If the right choice of weapon is made, this precondition can easily be met. However, there is also some skills and experience required to perform shooting.</td></tr>
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- the socio-economic aspects

Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).</td><td>No information available.</td></tr>
<tr><td>Level of confidence on the information provided [2]

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categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Rationale*: Although some shooting trials have been documented, the scientific evaluation of their effectiveness e.g. by application of population models to compare their efficiency with other methods, has yet to be determined.

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Measure description Provide a description of the measure, and identify its objective	**Introduction of predatory fish.** Biomanipulation by introduction of native predatory fish can have an impact on an American bullfrog population (Louette 2012). The method is also proposed for controlling crayfish and topmouth gudgeon *Pseudorasbora parva* populations (Lemmens et al. 2015), many of which species are on the Union List. Especially young pike *Esox lucius* will have a negative impact on the larval segment of an American bullfrog population (Louette et al. 2012b). Pike is a diurnal ambush predator that consumes an array of prey species and sizes, the choice primarily depending on prey availability and gape limitation (Craig 2008). It has proven to be an effective ecosystem engineer, altering communities in biomass and structure, provided stocking is conducted accurately (Skov & Nilsson 2007). It should, however, be noted that pike is considered introduced in some European countries (e.g. Ireland, Italy) (Carnevali et al. 2018; O'Flynn et al. 2018). Apart from pike as a pelagic predator, a number of other species could potentially be introduced for biomanipulation, such as pikeperch *Sander lucioperca*, perch *Perca fluviatilis*, eel *Anguilla anguilla* (Dörner & Benndorf 2003) or wels catfish *Silurus glanis* (Byers et al. 2006; Britton et al. 2011; Davies & Britton 2015), although data on the effectiveness of predation by these species on American bullfrog are largely lacking. Louette et al. (2012a,b) showed that the number of larvae in a pond where pike was introduced can be 10 times less compared to a similar pond without pike. To achieve these levels of larval reduction, a sufficient level of young pike needs to be maintained in the waterbody. Given the self-regulatory nature of pike, frequent restocking will most likely be required when natural reproduction is insufficient (Louette et al. 2012a,b; van Uytvanck & De Blust 2012). It is best to repopulate on a regular basis with young, six week old pike (5 to 10 cm in size). A minimum density of 500 individuals per ha is necessary to achieve effective biomanipulation (Louette 2012; Louette et al. 2012a,b; van Uytvanck & De Blust 2012). Introduction of pike will unlikely result in local eradication. However, the method can be used as post-treatment or as part of an integrated management approach, where habitat restoration by biomanipulation is combined with

<table>
<tr><td></td><td>reducing the impact of bullfrogs on native species (Devisscher et al. 2017). Pike also predate on other non-native fish species, such as pumpkinseed Lepomis gibbosus (Lemmens et al. 2015) and the Union List species topmouth gudgeon Pseudorasbora parva, which is sometimes also found in ponds with American bullfrogs. These non-native fish species frequently predate on macroinvertebrates like dragonfly and water beetle larvae, which are known to predate on bullfrog larvae. A reduction in macroinvertebrate predation levels will, therefore, also increase predation pressure on bullfrog larvae, further stressing them (Lemmens et al. 2015).</td></tr>
<tr><td>Scale of application
At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.</td><td>The method is suitable at the scale of small shallow lakes and ponds, as small pike is associated with vegetation, and larger lakes have much more pelagic habitat (Louette et al. 2012a,b; Lemmens et al. 2015). Experiments conducted in a complex of several small ponds (average surface area 1500 m^2) in Belgium showed significantly reduced levels of larvae in ponds where pike was introduced, in less than two years (Louette et al. 2012a,b).</td></tr>
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Rationale:
No full analysis of active biological control versus other management methods has been performed, but the method in itself has shown to be effective in reducing larval bullfrog populations (Louette et al. 2012a,b).</td></tr>
<tr><td>Effort required
e.g. period of time over which measure needs to be applied to have results</td><td>Experiments conducted in Belgium showed significantly reduced levels of larvae in treated ponds where pike was introduced, in less than two years (Louette et al. 2012a,b). However, because eradication is not a plausible outcome of this method, an end time cannot be determined. The only action required is periodical restocking with young pike brood.</td></tr>
<tr><td>Resources required [1]
e.g. cost, staff, equipment etc.</td><td>The cost of one restocking event is rather cheap, both in labour and actual cost of the brood. But when considering the long term nature of this method, the overall cost can become very expensive.</td></tr>
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species, etc. For each of the side effect types please select one of the impact categories (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	*Rationale*: This method can have both positive and negative effects on non-target species. Besides direct predation on bullfrog larvae, introduction of predatory fish also has indirect effects on the aquatic ecosystem through predation on bottom-dwelling and planktivorous fish (e.g. carp). This non-target predation can cause a reduction of nutrient levels in the water column which, in turn, reduces phytoplankton blooms. This renders the water less turbid and reduces available food for the American bullfrog tadpoles that primarily feed on periphyton, a mixture of algae, bacteria and detritus that is attached to submerged surfaces in aquatic ecosystems. In time, the clearer water also allows submerged water plants to flourish. The result is often an improved conservation status of the waterbody, despite bullfrog presence. On the other hand, pike is a gape limited generalist predator and can also predate on other, desired biota (e.g. fish, chicks of grebes and ducks, Dessborn et al. 2011). Other species like wels catfish *Silurus glanis* can also cause declines in other native fish species (Castaldelli et al. 2013). Although indirect effects of improved habitat quality can offset the negatives, the desirability of introducing piscivorous fish such as pike, perch or other species, requires an assessment based on the natural values present on a site, the status (native or not) of the introduced species and the recreational use of the waterbody for fishing, for example.
Acceptability to stakeholders e.g. impacted economic activities, animal welfare considerations, public perception, etc. Please select one of the categories of acceptability (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	***Acceptability to stakeholders*** | *Acceptable* | | *Neutral or mixed* | *X* | *Unacceptable* | *Rationale*: Pike do not limit their diet to American bullfrogs and non-native fish species, but will also predate on native, desired fish species, and vertebrates such as young or small birds e.g. little grebe (Louette et al. 2012a,b). In some cases, the desirability of pike introduction may be socially unwarranted, because of its non-native status, conflicting angling interests, or specific natural values with regard to endangered invertebrate, amphibian, fish or bird populations (Dessborn et al. 2011). The introduction of predatory fish might therefore be less accepted by certain stakeholders, such as anglers and birders.
Additional cost information [1] When not already included above, or in the species Risk Assessment. - implementation cost for Member States - the cost of inaction - the cost-effectiveness	No information available.

- the socio-economic aspects Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).	
Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Inconclusive* ☐ *Unresolved* ☐ *Established but incomplete* X *Well established* ☐ *Rationale*: The general cascading effects that piscivore engineers can have on aquatic communities through predation have been the subject of multiple field or experimental research projects, including meta-analysis (Skov et al. 2002; Dörner & Benndorf 2003; Hölker et al. 2007; Davies & Britton 2015). However, and although the amount of field studies dealing with the relationship between fish piscivores (mainly pike) and their accompanying (fish) community or specific target species (bullfrog, topmouth gudgeon) is extensive (Meijer et al. 1995, 1999; Skov et al. 2002; Louette et al. 2012a,b; Lemmens et al. 2015), only very few long-term standardised studies have been performed under natural conditions and even fewer have addressed the effect on American bullfrog tadpoles. The effect on bullfrog tadpoles is only known for pike.

Management - Measures to achieve management of the species once it has become widely spread within a Member State, or part of a Member State's territory. (cf. Article 19), i.e. **not** at an early stage of invasion (see Rapid eradication table above). These measures can be aimed at eradication, population control or containment of a population of the species. **This table is repeated for each of the management measures identified.**	
Measure description Provide a description of the measure, and identify its objective	**Sterile male release technique.** The sterile male release technique has been used to mitigate the impact of invasive alien species around the world, e.g. to control sea lamprey in the Great lakes region (Twohey et al. 2003; Bergstedt & Twohey 2005). American bullfrogs are very territorial. A single adult male will protect a stretch of shoreline, cannibalising other males that venture too close. Using this territoriality and cannibalism, sufficiently fit sterile adult males can have a significant impact on an American bullfrog population. The method induces a biased sex ratio in the population, by replacing a portion of the adult male population with sterile (triploid) individuals. To achieve eradication, probably at least 90% of the adult male population should be sterilised (Knipling 1959). Capturing or rearing this required number of individuals is difficult using current methods. Therefore, the method is probably, for the time being, more suitable

	for impact mitigation or as part of an integrated management strategy combining several control methods. Sterilisation of individual bullfrogs can be achieved either chemically (e.g. bisazir) or mechanically (e.g. pressure shock). However, while chemical agents and temperature shocks have been successfully used on different species, preliminary experiments to sterilise American bullfrogs eliminated the use of chemicals and temperature shocks, in favour of pressure shock treatment of fertilised eggs. Bisazir appeared hard to dose and had the potential of severe non-target effects, while temperature shocks yielded less success than pressure shocks (Descamps & De Vocht 2017). Additionally, the use of chemicals in aquatic ecosystems is prohibited in the European Union (Directive 2001/18/EC). The method described by Descamps & De Vocht (2017) requires for both males and females to be caught, by fyke netting or active trapping. The captured individuals are then harvested for eggs and sperm. The eggs are fertilised in the lab, treated with 6 minute long 5000 psi pressure shocks and reared until metamorphosis, before being released into the wild (Descamps & De Vocht 2017).
Scale of application At what scale is the measure applied? What is the largest scale at which it has been successfully used? Please provide examples, with areas (km^2 or ha) if possible.	The method is currently being tested in experimental conditions in Flanders (Belgium).
Effectiveness of the measure Is it effective in relation to its objective? Has the measure previously worked, failed? Please select one of the categories of effectiveness (with an 'X'), and provide a rationale, with supporting evidence and examples if possible.	***Effectiveness of measures*** — *Effective* [ ] — *Neutral* [ ] — *Ineffective* [ ] — *Unknown* [*X*] *Rationale*: Although successfully used on other aquatic biota, such as sea lamprey *Petromyzon marinus*, to control the invasive population in the Laurentian Great Lakes, USA (Hanson & Manion 1980; Twohey et al. 2003; Bravener & Twohey 2016), the method is currently still under development for American bullfrog and further testing is required to assess its effectiveness (Descamps & De Vocht 2017).
Effort required e.g. period of time over which measure needs to be applied to have results	The period of time over which the measure needs to be applied to be effective is at current unknown. More work is needed on this method before full scale implementation in the field can be considered.
Resources required [1]	The rearing of American bullfrogs and the production of triploid individuals in the laboratory requires specialised

<table>
<tr>
<td>e.g. cost, staff, equipment etc.</td>
<td>equipment and staff. However, because females produce large egg masses, harvesting a few females might be enough to produce large amounts of triploids.</td>
</tr>
<tr>
<td>Side effects (incl. potential) – both positive and negative
i.e. positive or negative side effects of the measure on public health, environment including non-targeted species, etc.

For each of the side effect types please select one of the impact categories (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td>
<td>
<table>
<tr><td>Environmental effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
<tr><td>Social effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
<tr><td>Economic effects</td><td>Positive</td><td></td><td>Neutral or mixed</td><td>X</td><td>Negative</td><td></td></tr>
</table>

Rationale:
Since sterilised American bullfrogs remain in the environment until they die, which can be up to 15 years (to be determined whether sterilised males can reach such age), a substantial predation pressure on native species will remain present in the environment. On the other hand, a reduction in the number of offspring, will reduce predation levels. When using chemical agents for sterilisation, the potential for non-target effects due to residues has to be considered.
</td>
</tr>
<tr>
<td>Acceptability to stakeholders
e.g. impacted economic activities, animal welfare considerations, public perception, etc.

Please select one of the categories of acceptability (with an ‘X’), and provide a rationale, with supporting evidence and examples if possible.</td>
<td>
<table>
<tr><td>Acceptability to stakeholders</td><td>Acceptable</td><td>X</td><td>Neutral or mixed</td><td></td><td>Unacceptable</td><td></td></tr>
</table>

Rationale:
Unknown, but the mechanical (non-transgenic) production of triploid, sterile individuals has the potential for being widely acceptable to stakeholders.
</td>
</tr>
<tr>
<td>Additional cost information [1]
When not already included above, or in the species Risk Assessment.
- implementation cost for Member States
- the cost of inaction
- the cost-effectiveness
- the socio-economic aspects

Include quantitative &/or qualitative data, and case studies (incl. from countries outside the EU).</td>
<td>No information available.</td>
</tr>
</table>

Level of confidence on the information provided [2] Please select one of the confidence categories along with a statement to support the category chosen. See *Notes* section at the bottom of this document. **NOTE – this is not related to the effectiveness of the measure**	*Inconclusive* X · *Unresolved* · *Established but incomplete* · *Well established* *Rationale*: Since the method is still in the experimental phase, no conclusions can be taken yet. To know more about the effectiveness of sterile male release technique to manage American bullfrog, an accurate estimation of the size of the target population and their population demography is necessary. Population models need to be applied to determine the optimal amount of triploid tadpoles to be released. Also, the catchability of adult bullfrogs needs to be improved, in order to harvest sufficient individuals for rearing and treating (Descamps & De Vocht 2017).

Inconclusive	X	*Unresolved*		*Established but incomplete*		*Well established*	

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See guidance section

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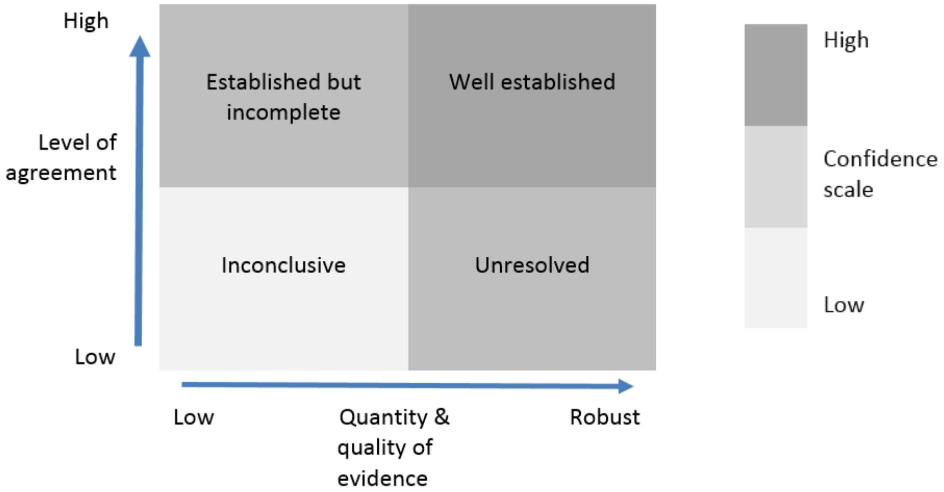
Notes

1. Costs information. The assessment of the potential costs shall describe those costs quantitatively and/or qualitatively depending on what information is available. This can include case studies from across the Union or third countries.

2. Level of confidence[1]: based on the quantity, quality and level of agreement in the evidence.



- **Well established**: comprehensive meta-analysis[2] or other synthesis or multiple independent studies that agree.
- **Established but incomplete**: general agreement although only a limited number of studies exist but no comprehensive synthesis and/or the studies that exist imprecisely address the question.
- **Unresolved**: multiple independent studies exist but conclusions do not agree.
- **Inconclusive**: limited evidence, recognising major knowledge gaps

3. Citations and bibliography. The APA formatting style for citing references in the text and in the bibliography is used.
e.g. Peer review papers will be written as follows:
In text citation: (Author & Author, Year)
In bibliography: Author, A. A., & Author, B. B. (Publication Year). Article title. *Periodical Title*, Volume(Issue), pp.-pp.

(see http://www.waikato.ac.nz/library/study/referencing/styles/apa)

[1] Assessment of confidence methodology is taken from IPBES. 2016. Guide on the production and integration of assessments from and across all scales (IPBES-4-INF-9), which is adapted from Moss and Schneider (2000).
[2] A statistical method for combining results from different studies which aims to identify patterns among study results, sources of disagreement among those results, or other relationships that may come to light in the context of multiple studies.