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

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

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Risk Assessment Area: The risk assessment area is the territory of the European Union (excluding the outermost regions) and of the United Kingdom.

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

Contents

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**[2]	**COMMENT**
Summarise Entry[3]	very likely	high	The species is already present in the risk assessment area via two main pathways (pet trade and research) which are still active. Facilities hosting captive populations are already present in several countries, hence the risk of entry is very high.
Summarise Establishment[4]	very likely	high	The species is already successfully established in the risk assessment area. There is also evidence that suitable conditions for the species are present in other countries where there are not yet established populations.
Summarise Spread[5]	rapidly	medium	Many studies have shown that the species may rapidly disperse by natural means (i.e. through ecological corridors) in the risk assessment area, facilitated by both the occurring environmental conditions and the species' intrinsic dispersal capacities.
Summarise Impact[6]	moderate	medium	*Xenopus laevis* can affect native species by competition and predation. In particular there is substantial evidence that it predates on amphibians (either eggs, tadpoles and adults), fish, and several

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

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

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

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

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

			groups of invertebrates, (particularly on macro-invertebrate communities), although there is no conclusive evidence of impact on their populations. Furthermore, there is evidence of *Xenopus laevis* potentially functioning as a reservoir for *Bd* and other pathogens. However, to date there is no evidence that *Xenopus laevis* has caused impact on native amphibians through pathogen transmission.
Conclusion of the risk assessment[7]	moderate	medium	The species is known to be invasive in the risk assessment area. Further releases, escapes (or spread) may occur in areas which are not yet colonised, leading to the successful establishment of new populations, hence increasing the overall impact associated with the occurrence of *Xenopus laevis* in the wild.

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

Distribution Summary:

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

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

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria				Yes	
Belgium	Yes		Yes	Yes	
Bulgaria				Yes	
Croatia				Yes	
Cyprus				Yes	
Czech Republic				Yes	
Denmark			Yes	Yes	
Estonia				Yes	
Finland				Yes	
France	Yes	Yes	Yes	Yes	Yes
Germany	Yes		Yes	Yes	
Greece			Yes	Yes	
Hungary				Yes	
Ireland			Yes	Yes	
Italy	Yes	Yes	Yes	Yes	Yes
Latvia				Yes	
Lithuania				Yes	

Luxembourg				Yes	
Malta				Yes	
Netherlands	Yes		Yes	Yes	
Poland				Yes	
Portugal	Yes	Yes	Yes	Yes	Yes
Romania				Yes	
Slovakia				Yes	
Slovenia				Yes	
Spain	Yes		Yes	Yes	
Sweden	Yes		Yes	Yes	
United Kingdom	Yes	?	Yes	Yes	

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine			Yes	Yes	
Atlantic	Yes	Yes	Yes	Yes	Yes
Black Sea				Yes	
Boreal				?	
Continental			Yes	Yes	
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian				Yes	
Steppic				Yes	

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

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

	• *Pipa laevis* (Daudin, 1802) • *Tremeropugus typicus* Smith, 1831 • *Xenopus boiei* Wagler, 1827 • *X. laevis* ssp. *bunyoniensis* Loveridge, 1932 • *X. laevis* ssp. *sudanensis* Perret, 1966
A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the wild, in confinement or associated with a pathway of introduction]	*X. laevis* is not difficult to distinguish from other anurans occurring in Europe (either native or alien ones). In general, the body of *X. laevis* has a flattened shape. Adult males measure around 90 mm in males, and females 100 mm, although larger individuals are known (John Measey pers. comm. 2018). The skin is smooth and slippery, with peculiar lateral lines along its sides. The eyes are positioned at the top of a small head, which lacks a tongue and eardrums. The hind legs are very developed and webbed (with black claws on the first three toes), while the front limbs are rather small. Colour varies from yellowish to olive grey or dark brown with spots (but albino forms are also common in trade). Tadpoles are easily distinguished from other (native) anurans, particularly because of their distinctive barbells next to the mouth, mid-water suspension feeding and often near transparent, especially when small. Other *Xenopus* species may be found in the trade. Examples are *X. tropicalis* and *X. epitropicalis*, which Tinsley & McCoid (1996) considered regularly imported to Europe with tropical fish from West Africa (although the source seems a bit outdated in this context). However, there is no evidence about the occurrence of such species in the wild in the risk assessment area.
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	Some risk assessments exist for the species, i.e. for Great Britain, USA, and Australia. In the risk assessment for Great Britain the risk attributed to the species is low, with a medium level of uncertainty (NNSS 2011). According to this assessment "*X. laevis* was having very minimal impacts in the UK, given the very few populations occurring in the wild" (which in fact are considered currently extinct, see details below), and the reduced ability to reproduce and further spread in the country (even if climate change would facilitate this). The only concern would be the unknown impact related to the possible spread of diseases (including the chytrid fungus) to native amphibians, and of possible unforeseen scenarios due to future climate

	changes. However the conclusions of the GB risk assessment cannot be extended to other EU regions, given the different climate and habitat conditions, which are definitely more suitable for the species, e.g. in the Mediterranean area. In the USA an Ecological Risk Screening Summary was made by the U.S. Fish and Wildlife Service (Anonymous 2017). The overall risk assessment category attributed to the species is "high", as the climate matched well in many states, and the certainty of the assessment was deemed medium (given the taxonomic uncertainties noted for the target species). Similarly, in Australia *X. laevis* has been assigned an establishment risk rank of "extreme" (Page et al. 2008). Since these assessments focus on the occurrence of the species in the US and Australia, their validity is limited by the difference in ecological, geographical and climatic conditions compared to the EU situation. Moreover, in the case of the US, the assessment should be revised in the light of the noted taxonomic uncertainties that are now resolved (John Measey pers. comm. 2018). A number of studies aimed at ranking the impact of amphibians were also carried out at either the global level (e.g. Kumschick et al. 2017a, Kumschick et al. 2017b, Kraus 2015, Measey et al. 2016, Measey et al. 2020) or EU level (e.g. Kopecký et al. 2016). For example, Kumschick et al. (2017b) discussed the application of the Environmental Impact Classification for Alien Taxa (EICAT, see Hawkins et al. 2015 for details on the methodology) on amphibians following two independent assessments made by Kraus (2015) and Kumschick et al. (2017a). The results showed that the impact classification is relatively "high" for *X. laevis*, despite some minor difference between the two assessments: respectively Massive (MV, irreversible community-level changes) and Major (MR, impact on a native community that is reversible). This difference seems justified by the practical interpretation and assignment of disease impacts in the absence of direct evidence of transmission from alien to native species, especially in relation to chytridiomycosis. The study was recently repeated (Measey et al. 2020) and the impact score for *Xenopus laevis* was confirmed as Major, while the confidence score changed from medium to high. It is also worth mentioning that the SEICAT assessment found little documented evidence for socio-economic impacts, except in the species native range where it can be a predator in aquaculture (Bacher et al. 2018).

	Measey et al. (2016) used the generic impact scoring system (GISS) to carry out a global assessment of alien amphibians. In particular *X. laevis* was the second top scoring amphibian for impact on native ecosystems (considering the sum for environmental and economic scores together) only after the invasive Cane toad *Rhinella marina*. Finally, it is worth mentioning Kopecký et al. (2016) who applied a risk assessment model (RAM) to ornamental amphibians traded in the EU. *X. laevis* was used as a reference species (together with *L. catesbeianus*) and was considered to have a moderate risk (the RAM value is 0.365), with an AmphISK invasion score of 10 (on a scale -10 to 33). This system however does not provide overwhelming evidence of risk, because as pointed out by the authors the RAM establishment value cannot be viewed as a precise estimation of the probability of establishment, but rather provides a relative ranking of ornamental amphibians traded in the EU. As a general remark regarding the scoring of the impact discussed above, it is important to consider that the categories used by this risk assessment (see Annex II) are different to those used by EICAT (according to which a major impact is reversible, contrary to the definition in Annex II which considers a major impact as irreversible). For example, as confirmed by John Measey (pers. comm. 2018) on the data used for this assessment made by Kumschick et al. (2017b), *X. laevis* was scored Major (within the EICAT scheme) on the basis of two studies (i.e. Lillo et al 2011, Grosselet et al 2005) both regarding predation, which suggest local extinction of native species (both were given medium confidence). However, the same impact under this risk assessment is considered as Moderate according to guidance in Annex II.
A4. Where is the organism native?	The full range of *X. laevis* covers much of southern Africa: South Africa, Lesotho, Swaziland, Namibia, parts of Botswana, Zimbabwe, parts of Mozambique and extending north into Malawi (Measey 2016, Ihlow et al. 2016). It means that native populations are distributed from winter rainfall regions in the south-western Cape region to summer rainfall regions in the north; and from sea level to 3,000 m in Lesotho (Measey 2004, De Busschere et al. 2016). As summarised by the Global Invasive Species Database (2015) *X. laevis* is a water-dependent species occurring in a very wide range of

	habitats, including heavily modified anthropogenic habitats. It lives in all sorts of water bodies, including streams, but tends to avoid large rivers, and water bodies with predatory fish. It reaches its highest densities in eutrophic water. It has very high reproductive potential. It is a highly opportunistic species, and colonizes newly created, apparently isolated, water bodies with apparent ease. *Xenopus laevis* exhibits high tolerance to salty water, pH variation (5-9, but there is evidence of the species breeding below pH 4, according to John Measey pers. comm. 2018), temperature variation (2-35+), and is capable of aestivation during dry periods. The species cannot spread naturally from its native range into the risk assessment area.
A5. What is the global non-native distribution of the organism outside the risk assessment area?	As reviewed by Measey et al. (2012) the global non-native distribution of the species is known to include four continents: North America, South America, Asia and Europe. In particular, established populations are present in different states of the USA, Chile, Japan. The species was recently recorded in China (Wang et al. 2019). There are historic records also for Mexico, Java, Israel, and Ascension Island, but with the notable exception of Mexico (where the species occurrence was recently confirmed, see Peralta-García et al. 2014) the presence of the species in these countries is not confirmed. Regarding Europe, see details under points A7-A9 below.
A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established?	The species was recorded and is established in both the Mediterranean and Atlantic biogeographic regions (see details in A8).
A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change?	Current climate conditions: Mediterranean, Atlantic, Continental and Alpine biogeographic regions Foreseeable climate change conditions: all biogeographic regions, with the exception of the Boreal and Arctic, may be suitable (at least in part) depending on the different models used (see ANNEX VI). However the confidence level is very low, as we were not able to retrieve precise information on future climate, given the methodological constraints and the lack of accurate information available on the species location and taxonomy. For details on the assumptions

	made in relation to climate change see annex VI: projection of climatic suitability. Recent studies have shown that native phylogeographic lineages have contributed differently to invasive *X. laevis* populations, but most of the introductions have probably been from the Mediterranean climate zone in the southwest of the Western Cape Province, South Africa, where *X. laevis* occurs naturally. For example, according to genetic and historical data the populations established in Europe, and in particular in Italy (Sicily), Portugal and France, seem to involve individuals from the south-western Cape region in South Africa (De Busschere et al. 2016, Lillo et al 2013). In France however another distinct native phylogeographic lineage is involved, i.e. from other regions of South Africa (De Busschere et al. 2016, Rödder et al. 2017). The identification of source populations is particularly relevant for the purpose of this document, because phenotypic as well as genotypic traits of colonizing individuals might influence the invasion process and success, particularly in such cases where there is extensive population differentiation within the native range (De Busschere et al. 2016). To assess the future distribution under current climate, Measey et al. (2012) used a single lineage of the species from the southwestern Cape of South Africa for their species distribution models (SDMs). As a result, the optimal uninvaded bioclimatic space was identified in isolated parts of France and Portugal only, while a large suitable climatic potential was identified for most of southern Portugal and adjoining Spain, as well as central and southern France, and mainland Italy. Such data are consistent with the finding of Ihlow et al. (2016), who used the entire range as well as invasive populations and who predicted particularly high probabilities in Europe, namely in Portugal, eastern Spain, southern France, and Italy. Furthermore, Ihlow et al. (2016) highlight areas in Spain (including the Balearic Islands), mainland Italy (including Sardinia), and southern France (including Corsica) to be highly vulnerable to potential invasions, as these regions exhibit suitable climatic conditions for *X. laevis* and are adjacent to established invasive populations. According to Measey et al. (2012) a few suitable areas were found also in the United Kingdom outside southern coastal areas, plus Greece, Ireland, Germany, Belgium, Denmark and the Netherlands (for details see maps developed by Measey at al. 2012). On the other hand, Ihlow et al. (2016) predict only moderate probability for Great Britain, where populations from Wales and Lincolnshire have recently been extirpated. Therefore, while the optimal area would fall within the Mediterranean and Atlantic

	biogeographic regions only, the maps annexed to the study seem to suggest the presence of suitable areas also within the Continental and Alpine regions. Under foreseeable climate change, using species distribution models (SDMs), Ihlow et al. (2016) assessed the global invasion potential of this species for 2070 following four IPCC scenarios (i.e. RCP2.6, RCP4.5, RCP6, RCP8.5). In particular, the potential range size was predicted to expand in north-western Europe, especially in France and Great Britain, where new regional conditions may promote new invasions or the spread of established invasive populations. The Mediterranean area was already considered suitable and still is under all climate change scenarios. The maps shown in the paper by Ihlow et al. (2016) do not allow for a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions in Europe will become suitable for the species. Rödder et al. (2017) demonstrated that invasive populations of *X. laevis* are established well beyond the species' multivariate realized niche in southern Africa. Hybridization of different lineages may have enabled a shift in the species' fundamental niche. Given the magnitude of the detected niche shifts, the usefulness of climate matching approaches to assess invasion risk for this species is challenged, as it might frequently underestimate the true potential distribution when a geographic subset of the species' realized distribution is used for model training. It can be expected that the true invasion potential for *X. laevis* is larger than its estimated potential distribution based on its currently realized niche (Rödder et al. 2017). Similarly, in a recent study, Ginal et al. (2020) stressed that *X. laevis* is a species with a strongly underestimated invasive potential. They point out that the correlative approaches which characterized the previous SDMs (Ihlow et al., 2016; Measey et al., 2012, Rödder et al., 2017) can be vulnerable to extrapolation errors when projecting species' distributions in nonnative ranges. Therefore, to better assess the species' full invasive potential, the authors developed a process-based model based on physiological data like critical thermal limits and temperature-dependent performance. The study suggested a high risk of invasion for most parts of Europe that could be assessed. In particular, according to Ginal et al. (2020), large parts of western and southern Europe as well as many Mediterranean islands are predicted as being climatically

	suitable for *X. laevis*. This also includes the currently (or formerly) occupied areas of Portugal, France, and Wales. Sicily is predicted as unsuitable, which the authors state is probably due to the low density of waterbodies, preventing assessment by their waterbody availability layer. Extensive "MESS" areas, which highlight possible extrapolation errors, occur in central to eastern Europe as well as in cold mountain ranges such as the Alps and Pyrenees, where predictions should be treated with caution.
A8. In which EU member states has the species been recorded and in which EU member states has it established? List them with an indication of the timeline of observations.	Recorded in the following Member States: Belgium, France, Portugal, Italy, Spain, Sweden, Germany, Netherlands; and in the United Kingdom. Established: France, Portugal, Italy. In general, the situation has been quite dynamic in the last years, mostly due to the fact that introductions are usually followed by a lag of around 15 years between the export of animals and the rise in invasive populations (van Sittert and Measey 2016). In general, it can take between 2 and 25 years or more for first reports of introductions to be released (Measey et al. 2012). As discussed in detail below, the species is currently considered established in France, Portugal and Italy (Sicily). Until recently, the species was considered established also in the UK, where it is currently considered extinct. According to Frazer (1964) the first introduction in the UK occurred in Kent in 1955, but did not succeed. The UK was also home of the first invasive population established in Europe, namely on the Isle of Wight, due to an introduction around 1962 (Tinsley et al. 2015a, Tinsley & McCoid, 1996, van Sittert and Measey 2016). In the UK, in addition to the population on the Isle of Wight, now probably extinct, there have also been two established populations, namely in Glamorgan (Wales), and Lincolnshire (England). They were both the subject of an eradication programme and are considered recently extinct, although the causative factors were possibly the exceptional weather conditions (in conjunction with specific habitat and population characteristics) (Tinsley et al. 2015a). In any case, follow-up monitoring is still required in South Wales (John Measey, pers. comm. 2018). The species was also reported in 1987 and 1990 in two ponds to the southeast of London, although these do not appear to have survived,

	and in the southwest of England, but no established populations have been found (see review made by NNSS 2011). In Portugal the species was first found in 2006 and first reported in 2007, but the first introduction may have occurred in Oeiras in 1979 (Sousa et al. 2018). In Italy, the only known population of *X. laevis* is on the island of Sicily where the date, site and cause of first release are all unknown (Measey et al. 2012). The first documented occurrence dates back to 1999, while the first report was in 2004. In peninsular Italy, the presence of two adults of *X. laevis* was reported in 2017 for the Lombardia region, in the Groane park (see http://www.parcogroane.it/wordpress/wp-content/uploads/2017/08/Report-censimento-anfibi-Progetto-LIFE-GESTIRE-2020-1.pdf). In France animals were officially first reported in 1998. However, residents of the area suggested that this frog had been present since the early 1980s (Fouquet 2001, Fouquet and Measey 2006, Measey et al. 2012). A new population of *X. laevis* has been discovered in Toulouse in 2018 (see http://especes-exotiques-envahissantes.fr/decouverte-xenope-lisse-occitanie/). As reported on the mentioned link, this discovery brings to four the number of known sites of occurrence of *X. laevis* in France. Indeed, in addition to the main nucleus located in between four departments (Deux-Sèvres, Vienne, Maine-et-Loire, Loire-Atlantique), a population was discovered in 2015 in Ambarès-et-Lagrave, near Bordeaux, then another one in La Chapelle-d'Armentières, near Lille, in 2018. The latter population is close to the border with Belgium, where an individual was already reported in 2006 (see below). In Spain, the presence of *X. laevis* was reported in Montjuïc (Barcelona) in 2007, where about 30 larvae were found. A year later, six individuals were found in the Parc del Laberint d'Horta. Both the larvae and the adults found were removed from the environment (EXOCAT database http://exocatdb.creaf.cat/base_dades/#), and the species is now considered eradicated (Pascual et al. 2007). In Sweden, a single animal exists in the collection of the Gothenberg Natural History Museum collected in 2007 (Measey et al. 2012). However, this record is not reliable as the locality

	reported by Measey et al. (2012) is in fact a name of a person who received a dead frog from another person (Melanie Josefsson in litt. 2018). In Germany, *X. laevis* specimens have been collected in the Hamburg area, following a release in 1991 by animal rights activists (Tinsley and McCoid, 1996; Rabitsch et al., 2013). Their current status is unknown, but it is very likely that they have disappeared. In the Netherlands there are records of an adult individual caught near Gorichem in 1974 and of tadpoles collected near Utrecht in 1979 (Tinsley and McCoid, 1996). The National Database Flora and Fauna has no recent records for the species in the Netherlands, confirming that the species has not established populations (www.ndff.nl, see also https://www.verspreidingsatlas.nl/soortenlijst/amfibieen). In Belgium, as reported in the Hyla (amphibian and reptile working group of the ngo Natuurpunt) database, 30 *X. laevis* larvae were found at Antwerp University pond in 2008 (observer Bart Vervust). Their current status is unknown. Two other individuals were found in Ploegsteert (Wallonia) as reported on observations.be, one in 2006 (an albino specimen) and one in 2016. The area is at the border with the French area where in 2018 a new population of the species was reported, as stated above. However, an eDNA study currently in progress has confirmed that no population occurs on the Flemish side of the border (Van Doorn et al. 2021, unpublished report). According to Measey (2017) wild caught *X. laevis* are also reported to have been imported into the USA from the Czech Republic and Switzerland over the period 1999-2005. Such records are however presumed anomalies and should be treated with suspicion, as no invasive populations are known in these countries. On the other hand, as the source of information are records from USFW for animals imported into the USA, they could be specimens caught in the wild in South Africa and then exported to USA via the Czech Republic or Switzerland (John Measey, pers. comm. 2018).
A9. In which EU member states could the species establish in the future under current	Current climate conditions: France, Portugal, Italy, Spain, Sweden, Germany, Netherlands, Greece, Ireland, Belgium, Denmark; and the United Kingdom

climate and under foreseeable climate change?	Foreseeable climate change conditions: all EU countries may be suitable (at least in part) depending on the different model used (see ANNEX VI). However, the confidence level is very low, as model projections for the current climate vary widely, and projections for future climates are not available for all models. We could not retrieve precise information on future climate, given the methodological constraints and the lack of accurate information available on the species location and taxonomy. Ginal et al. (2020), in their distribution model paper, did not include future climate scenarios. For details see comments on point A7.
A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?	There is evidence of invasiveness in the USA, Chile and Japan (see section on impacts below for more details).
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	The species has shown signs of invasiveness in both the Mediterranean and Atlantic biogeographic regions, i.e. in all areas where populations are established. For details see section on "magnitude of impact", points 2.13-2.30 below.
A12. In which EU member states has the species shown signs of invasiveness?	The species has shown signs of invasiveness in all EU Member States where populations are established, i.e. France, Portugal and Italy (Sicily).
A13. Describe any known socio-economic benefits of the organism.	*Xenopus laevis* is a species substantially important in relation to research activities in the risk assessment area and globally (amongst others in oncology, endocrinology, developmental biology and anatomical studies), see e.g. Gurdon and Hopwood, 2000, Hardwick and Philpott, 2015 and Blum and Ott, 2018). The species was used to diagnose pregnancy, but this is apparently no longer a practice in the risk assessment area, and we may exclude its use for this purpose in other parts of the world too. The species is also traded as pet for aquaria and garden ponds. **Pet trade** In relation to the pet sector, sales and associated ancillary product sales of *X. laevis* are significant, particularly in UK.

	According to data provided by the Europen Pet Organization to the European Commission (EPO 2018), in the UK, the annual revenue for the pet sector from *X. laevis* is in the estimated range of between 168,500 euros to 3 million euros but they consider this is likely to be a conservative estimate. This species is also likely to be economically important to other Member States where trade in this species (as a pet) is permitted, although EPO acknowledges that this may be to a lesser degree when compared to the UK. Furthermore, EPO consider that an albino morph of *Xenopus laevis* is dominant in the pet trade. More in detail, according to EPO (2018) from the data from those Member States where *X. laevis* is traded by the pet sector, individual animals are sold for values between 1 euro to 11,30 euros depending on the Member State. Therefore, in terms of the total trade based on the information collated by EPO, between the different countries there is a very broad range in the trade values of this species (based on the number of individuals sold), with numbers ranging from a lowest value of 275 euros per annum to as high as 1 million euros per annum. If ancillary products e.g. aquariums, dry goods etc. are factored in, this figure is significantly higher with a conservative estimate of 3 million euros per year across EU and UK. While EPO (2018) does not provide a breakdown per country, they clarify that *"the proportion of sales of animals (and therefore ancillary products) varies significantly between Member States, with the UK representing the highest values whilst those reported for the Netherlands and France being significantly lower"*. Due to the skewed nature of the raw data (which EPO was unable to provide given that it is highly commercially sensitive), EPO was unable to provide median or average estimated trade revenues. However, EPO noted that the figures provided are likely to be a conservative estimate. In the UK, the annual revenue from *X. laevis* for the pet sector could be anywhere between 168,500 euros to 3 million euros. This represents a very broad range, which means that banning this species could have an impact for pet stores related activities in some EU countries. The species is available for purchase within the risk assessment area also on the internet, e.g. https://www.siervissen-onlineshop.be/fr/shop/grenoulle-apprendre-plus/ https://www.oxyfish.fr/especes-variees/33346-xenopus-laevis-albino-1-5-2-cm.html https://www.ebay.co.uk/itm/Albino-African-Clawed-Frog-Xenopus-Laevis-/264926197064

	(accessed on 05/02/2021). **<u>Research activities</u>** In relation to the use of the species in research activities, this frog had a significant role in the history of 20th century science. It became one of four vertebrate species universally recognised as representing a standard biological model (van Sittert and Measey 2016, Gurdon and Hopwood, 2000). EXRC (2019) indicate that more than 38000 papers are found by a search for "*Xenpous laevis*" in the online archive of biomedical scientific publications Pubmed. Research based on *Xenopus laevis* includes the work by Sir John Gurdon, awarded in 2012 with the Nobel Prize in Physiology or Medicine[8]. The use of the species in research activities in the EU is regulated by Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes[9]. According to this, specimens of *Xenopus laevis and Xenopus tropicalis* may only be used in procedures where those animals have been bred for use in "procedures". Animals taken from the wild shall not be used in procedures but exemptions may be granted to this rule. "Procedure" means any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. Furthermore, Member States shall ensure that all breeders, suppliers and users are authorised by, and registered with, the Member State's competent authority and keep records of number, origin, source etc. of animals. Statistical data covering the EU 28 Member States (EU28) data over the period 2015-2017 were published in early 2020[10]. These statistics cover both *X. laevis* and *X. tropicalis* (data is

[8] https://www.nobelprize.org/prizes/medicine/2012/gurdon/facts/

[9] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02010L0063-20190626&from=EN

[10] https://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

	collected for both species together). According to these, the number of "all uses" for these two species, i.e. the number of times these species were needed for scientific purposes in a given year in EU28 (first use and any subsequent reuse) were: • in 2015: 18,990 uses of which 42.9% were reuses • in 2016: 27,904 uses of which 33.70% were reuses • in 2017: 21,443 uses of which 36.90% were reuses For the purpose of the above, the "use" of an animal within a project extends from the time the procedure (or first procedure/technique in a series) is applied to it, to the time when the observations, or the collection of data (or other products) for a particular scientific purpose (usually a single experiment or test), are completed. "Reuse" indicates any subsequent use of an animal, which has already completed a procedure (or series of procedures/techniques) for a particular scientific purpose, and for which any animal would suffice. Reuse of *Xenopus* is relatively high. "All uses" (first use and any subsequent reuse) indicate the number of times these species were needed for scientific purposes in a given year in the EU. EXRC (2019) estimated on basis of a literature review carried out a decade earlier that somewhere between 300 and 400 labs across Europe rely on *X. laevis* for their work. They furthermore estimate that the population of *X. laevis* used for developmental biology alone is over 52,000 animals based on unpublished survey data of 210 laboratories worldwide. Based on contributions from scientists using *Xenopus* in their research, affiliated to 22 Universities and research institutes in Belgium, Denmark, France, Germany, Ireland, Italy and the United Kingdom, EXRC (2019) also indicated that these scientists had in 2019 32,55million EUR in grant income, supporting over 100 early career-scientists. Furthermore, in a given academic year in these 22 institutions over 600 students would be taught fundamentals of cell and developmental biology using *X. laevis*. EXRC (2019) consider that for most of the experiments that use *X. laevis*, there are no alternatives (e.g. egg extracts, oocyte-based receptor assays, developmental biology using traditional techniques), for genetic approaches to cell and developmental biology *X. tropicalis* can be used but the investment needed for labs to change to this species would be very considerable. EXRC (2019) also consider that research using *Xenopus* contributes very strongly to the

	Commission and Society's aim to replace the use of mammals such as mice in biological and biomedical experimentation (the so-called 3Rs of reduction, refinement and replacement). The use of Xenopus in place of mice is a refinement of experimental design that supports Directive 2010/63/EU. Biomedical research has resulted also to commercial use of *Xenopus* embryos and tadpoles for in vivo drug discovery purposes and the technology has been protected by European and US patents (EP2409149, US Patent 9945845). In addition to its widespread role in biomedical research, *X. laevis* is used for testing environmental contamination and the teratogenic potential of new products. These tests are usually carried out commercially and examples include water testing by "Watchfrog" (https://www.watchfrog.fr) and the well-established "Frog Embryo Teratogenesis Assay-Xenopus (FETAX)" which is routinely used since early 2000's as a developmental toxicity screening test for pharmaceutical candidate compounds (Leconte and Mouche, 2013). According to EXRC (2019), most laboratories import animals either from the CRB Xenopus in Rennes, NASCO in the US or the EXRC; only a few breed their own. Indeed, within the risk assessment area, specialised suppliers of *Xenopus laevis* (and other *Xenopus* species) exist in the risk assessment area, e.g. in France and UK. See for example http://www.xenbase.org/other/obtain.do, https://xenopusresource.org/how-to-order and https://xenopus.univ-rennes1.fr/tarifs-0 (assessed on 05/02/2021). Data from Measey (2017) show that there are some imports into the US from other countries with known invasive populations (Chile, UK and France), but in small quantities (< 100 animals), and of captive bred animals usually for medical or scientific purposes and are thus presumably not from invasive populations. Animals in France were being harvested by Xenopus Express in France, although it is unknown whether or not this practice continues (John Measey, pers. comm. 2018). According to trade data of the U.S Fish and Wildlife Service presented in Measey (2017), live individuals of *X. laevis* were imported into the USA from EU countries such as Czech Republic, Germany, and Italy (therefore, including countries without known populations established in the wild, like Czech Republic and Germany). This could involve re-exports (see also section A8 on this).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: "No information has been found."
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[11] and the provided key to pathways[12].
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of introduction and entry.

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

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

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

pathways respond N/A and move to the Establishment section)			et al. 2010). The main active pathways are therefore the following: 1) Pet/aquarium/terrarium species (escape from confinement); 2) Research and ex-situ breeding (in facilities) (escape from confinement). As pointed out by Tinsley & McCoid (1996), it may be due to many factors, such as loss of interest, end of an experiment, misguided ethics or curiosity, which occasionally results in the release of captives. Deliberate release are also reported, along with escapes (Measey et al. 2012). The actual scale of releases and escapes is unknown. In fact, in most cases the exact cause is only inferred retrospectively, as the species is often detected only many years after its deliberate or accidental introduction. For example, in Portugal the species lived undetected for more than 20 years (Sousa et al. 2018). In such cases, it is clear that it is not possible to establish the intentionality of the introduction without the relevant events being appropriately documented.
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (copy	1) Pet/aquarium/terrarium species (escape from confinement) 2) Research and ex-situ breeding		In addition to the use of the species as a research model in laboratories and as a pet (which leads to the main two active pathways already identified), other uses are known. For example, the species has been used in schools for training in labs (e.g. dissections etc.),

and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	(in facilities) (escape from confinement)		which can be a source of animals released in the wild. While there is no documented evidence of such releases in Europe, in the US, schools are known to ditch their stock when legislation changed making keeping invasive species illegal. The law changed without making any provision for people already keeping them (John Measey, pers. comm. 2018). In any case, the use of animals in schools is treated here under the pathway "Research and ex-situ breeding (in facilities)", see point 1.4b below. As reported by Weldon et al. (2007) in South Africa *X. laevis* is appreciated as live bait for freshwater angling (despite this practice being illegal). As a consequence, fishermen are known to seed dams with *X. laevis* in order to produce a local supply of live bait (Measey et al. 2017). However, this is not considered an active pathway in Europe. Zoo exhibit of this species is also mentioned as a former pathway in the late 1900s (Vredenburg et al. 2013), and although the species is still present in public zoos/aquaria, zoos (escape from confinement) are not considered as an important pathway of introduction of the species in Europe. Whilst there is no data available on the total population within all zoological collections in the EU, information was provided by EAZA (European Association of Zoos and Aquaria) on populations kept at approximately 300 of their

			Member zoos and aquariums in 26 EU Member States (with the exceptions of Cyprus and Malta). The information provided by EAZA (EAZA, pers. comm. 2018) indicates that 84 specimens in total are kept by 15 zoo/aquarium EAZA Members in 10 Member States (BE, HU, DK, NL, IE, DE, PT, PL, EE, FR) and the United Kingdom. On top of this, in total 5 specimens of the subspecies *Xenopus laevis* are kept by 1 zoos/aquariums EAZA Members in one Member State (CZ). This data comes from the animal care and management software provided by Species360 Zoological Information Management System (ZIMS) (zims.Species360.org, 2018) whose usage is widespread throughout the EAZA Membership. It must be noted that the actual situation might slightly differ if the species has been recorded under a different/older taxonomic name (as in the case of *X. laevis*).
Pathway name:	**1) Pet/aquarium/terrarium species (escape from confinement)**		
1.3a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?	**intentional**	**high**	*X. laevis* is traded by the pet sector in several Member States of the EU as well as in UK, with the albino morph being predominant in this trade. The species is also traded through e-commerce

(if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows[13])			and other internet platforms (although numbers are unknown). The introduction in the risk assessment area through this pathway is intentional. However, the entry into the environment is either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes. Despite the general lack of documented evidence regarding the significance of this pathway for the entry of the species into the environment, there are indications that this pathway is active in Europe, as well as in other parts of the world. For example, according to Measey et al. (2012) the source of a population once occurring near Scunthorpe, Humberside, in the north-east of England, is thought to be due to the closure of a pet shop and the deliberate release of adults in the mid-1990s. EPO-OFI (2019) on the other hand consider that there seems to be no substantive evidence that the closure of the pet shop led to a deliberate release or that the speculated pet shop ever kept *Xenopus*. They consider it possible that the population in question existed prior to the closure of this shop. The trade and possession of the species is not regulated in most Member States and EPO

[13] While the pathways of introduction are considered intentional, it was considered necessary to answer all questions since the subsequent entry into environment could be either intentional or unintentional.

			(2018) reported that trade is ongoing between different countries in the risk assessment area (the Netherlands, Czech Republic) and the UK. On the other hand, the species is regulated in other Member States, subjecting its trade and/or possession to diverse national measures, e.g. • Possession and trading of *X. laevis* is prohibited in Spain (Real Decreto 630/2013, de 2 de Agosto[14]). • In Portugal, the species is included in the national list of invasive alien species under the Decree-law of 1999 and subsequently that of 2019[15]. • In France, the release of the species into the environment is forbidden[16]. Keeping the species requires an authorisation and a certificate of competence (though this is not limited to professionals) and it is prohibited to exhibit the species for sale [17]. In Chile there is evidence of continuous releases (meaning that animals were moved from within the invasion area to a new area) which helped the species spread, and which are presumed to be connected with the use of animals in the pet trade, as private owners were suspected of dumping them (Lobos & Jaksic 2005, Measey et

[14] https://www.boe.es/buscar/act.php?id=BOE-A-2013-8565
[15] Decreto-Lei n.o 565/99 de 21 de Dezembro and Decreto-Lei n.º 92/2019 de 10 de julho
[16] Arrêté du 14 février 2018 propagation des espèces animales exotiques envahissantes sur le territoire métropolitain – Annexe I
[17] Arrêté du 8 octobre 2018 fixant les règles générales de détention d'animaux d'espèces non domestiques – Article 14

			al. 2012). Also in the USA, there is evidence of animal importer dumping unwanted stock, e.g. in Florida (King and Krakauer 1966), or intentionally released by a single person, in Arizona (Somma 2018, Tinsley and McCoid, 1996).
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.	**very likely**	**medium**	In general, information on the origin of animals, exact number of individuals in trade and those in captivity, is not available. Also, no information could be retrieved on number of introduction events and number of individuals (either adults or larvae) escaped and/or released in the environment, hence it is not possible to assess the propagule pressure. However, according to the European Pet Organization (EPO 2018) in relation to animal traded in the UK, *X. laevis* is captive bred and none are wild caught. The extent of breeding in other EU Member States is unknown. In terms of numbers traded the data provided (EPO 2018) suggest a widespread range between the different countries with the lowest value being 100 animals sold per annum to the highest value of tens of thousands of animals being sold per annum. Therefore, due to this wide range of values, EPO was unable to provide median or average numbers/volumes due to the skewed nature of the raw data. EPO did not provide the raw data given that this information is commercially sensitive. It is however evident that

			the highest numbers concern the trade within UK. Information from other regions may help to figure out the dimension of the problem although the pet trade markets in the USA and Europe may not be directly comparable. As regards the USA, it is worth mentioning that across the last decades *X. laevis* trade has changed dramatically in terms of primary purpose, frogs' origin and numbers of animals traded. The species was originally distributed for pregnancy testing and laboratory use, but in the last 15 years, the size of the trade for medicine and science dropped to only 0.1% of imports, with the pet trade commanding 99.6% (Measey 2017). Trade figures reported by Measey (2017) for the USA are impressive (see also Herrel and van der Meijden, 2014). Whilst trade for medical and scientific purposes is now minimal (a few hundred animals per year), the pet trade imported 1.83 million live animals over the last 15 years (a total of 1,856 shipments which ranged from single animals to 11.5 thousand individuals). Just to give an idea of the global trade network supporting such trade, 75% of these animals are imported from Hong Kong (although it is possible that many animals originate from mainland China or elsewhere). It is also worth noting that all of these animals from China appear to be albinos, and there are currently no published reports of invasive albino populations, despite a single exception recently found in China (John Measey, pers. comm. 2018,

			Wang et al. 2019). Only 5,600 animals were imported from the native area in South Africa, and this trade ceased in 2003. Nearly 200,000 individuals were imported from Chile and the majority of these were reported as being wild caught, suggesting that the invasive population there is being exported for the US pet trade (Measey 2017). Given the supposed widespread presence of this species in the pet trade in several countries, the risk of reinvasion after eradication is to be considered as likely as a first introduction. There are no specific studies providing an indication of the propagule pressure, but single gravid females can indicatively contain from 1,000 to 27,000 eggs per clutch (noting though that the number of 27,000 is not the norm, numbers are usually much less). Furthermore, females will produce multiple clutches in a season under favourable conditions (Global Invasive Species Database 2015), therefore even a handful of individuals may be sufficient to start a new population. In fact, as shown by Lobos et al. (2014), the invasion of *X. laevis* in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population. Also according to Measey et al. (2012) propagule pressure plays a pivotal role in the establishment of *X. laevis*, as some

			populations became established after the release of large numbers of animals from breeding facilities (laboratory and pet supplies). Other evidence of populations that have established from very few individuals is not available (John Measey, pers. comm. 2018).
1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**very likely**	**medium**	The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been frequently traded and that the origin of some populations successfully established after animals being released in (or escaped into) the wild is attributed to this pathway.
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	**very likely**	**low**	No information has been found. Trade is intentional, and as such there is no management practice in place to prevent the species entering the risk assessment area. Also, there are no known specific practices for preventing this species from escaping or being released in the wild. Existing biosecurity guidance concentrates in the prevention of disease transmission when species are transferred (OATA 2012).
1.7a. How likely is the organism to enter the risk assessment area undetected?	**very likely**	**high**	While animals intentionally introduced in the risk assessment area for the pet trade are not undetected, those being introduced in the wild as a consequence of accidental escapes or intentional releases can be undetected for many years (see point 2.7a below)

1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**moderately likely**	**medium**	We are not certain whether any particular time of the year is more appropriate for establishment. It is likely that *X. laevis* could establish during any month of the year. In any case, traded animals may arrive and be released or escape at any time during the year in Europe, but data about frequency and months of the year are unknown.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**moderately likely**	**medium**	It is likely that people who deliberately release *X. laevis* into the wild will do it in what they consider the most suitable habitat. As a remark, *X. laevis* is a vigorously adaptable species which may virtually inhabit any type of water bodies, including lakes and rivers, as well as permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides *X. laevis* thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005). This clearly increases the likelihood of the species being introduced, either intentionally or accidentally, into suitable habitats. There is also a high likelihood that additional translocations by humans within the risk assessment area may occur (see also Qu. 2.1 and 2.2), hence increasing the opportunities for the species introduction within the risk assessment area. In addition, as pointed out by Measey et al. (2012) biosecurity at breeding facilities is clearly

			of paramount importance. It is reasonable to assume that maintenance staff in pet wholesaler/retailer premises may have received in-house training, as also suggested by EPO 2019. Furthermore, codes of conduct (e.g. Council of Europe, 2011) call for promoting awareness of invasiveness and of the need not to release pets in the environment in general. However, care sheets addressed to keepers of *X. laevis* as pets available in the public domain concentrate on animal welfare considerations with very little or no information regarding the prevention of escapes and in any case with no advice against releases into environment and the potential environmental impacts of such releases (e.g. Reed 2009, amphibian.co.uk).
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**very likely**	**high**	In current conditions, it is very likely that the species will enter the environment within the risk assessment area through this pathway. The species is known to be present in the pet trade in Europe, and has already been recorded in the wild in the region, possibly also as a consequence of this pathway.

Pathway name:	**2) Research and ex-situ breeding (in facilities) (escape from confinement)**		
1.3b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?	**intentional**	**high**	The species is traded as model amphibian in scientific research, and as such the introduction in the risk assessment area through this pathway is intentional. However, entry into the environment is

(if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)			either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes. Despite the general lack of documented evidence regarding the exact pathway of introduction for this species, there are clues of this pathway being active in Europe, as well as in other parts of the world. For example, in France the suspected origin of the species was a breeding facility of the CNRS in Fronteau, Bouillé St Paul (Fouquet 2001), a laboratory supplier for French research institutions (Measey et al. 2012). In Portugal, the species was likely introduced following a flood of the 1979/1980 winter in the laboratories, where the species was used, although this is unconfirmed (Rebelo 2010, Sousa et al. 2018).
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.	**very likely**	**high**	There is evidence linking the occurrence of invasive alien populations with the trade and use of this species for biomedical research, although other secondary pathways seem to be involved as well (van Sittert & Measey 2016). The history of the use and trade of the species started in the 1930s with the use in pregnancy testing until the 1960s, and later for laboratory use as model organism (Gurdon and Hopwood 2000, Measey et al. 2012, Tinsley et al. 2015a, van Sittert & Measey 2016). This led to exports of thousands of live animals from its native South African Cape region to laboratories, first to the United Kingdom and eventually all over the world. By 1970, as

			demonstrated by van Sittert & Measey (2016), *X. laevis* was the world's most widely distributed amphibian: institutions in 48 countries were supplied with live animals on all continents except Antarctica. In fact, as summarized by Weldon et al. (2007), the use of this species as a model amphibian in scientific research (i.e. genetics, molecular biology, embryology, biochemistry and ecotoxicology) was increasingly popular in the 1970s, and *X. laevis* became the most widely used amphibian in research in the 1990s. In terms of numbers, over 10,000 animals were exported annually from South Africa between 1998 and 2004 to 132 facilities situated in 30 countries (Weldon et al. 2007). However, there is still no known information on how many animals were shipped privately and where they were shipped to, during this period. Additionally, the secondary movement between places that were supplied also appears to be important (John Measey, pers. comm. 2018). *X. laevis* was also used for educational and training purposes in schools and universities (e.g. dissection classes). However, this use seems declining markedly due to ethical concerns and financial constraints (Reed 2005). Of note here is the link between tadpoles and home teaching: in many cases, the tadpoles are reared in large numbers and then many are euthanized. Some individuals will give tadpoles over to parents to

			raise them at home, or liberate tadpoles. This is less likely in the pet trade, but may happen in tertiary education institutes where *X. laevis* is a teaching model (John Measey, pers. comm. 2018). Given the widespread presence of this species in research facilities in several countries (see section A13), the risk of reinvasion after eradication is to be considered as likely as a first introduction.
1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**very likely**	**medium**	The species is able to survive during passage along the pathway. Actually, specimens are intentionally transported for use in laboratories. According to EXRC (2019), most laboratories import animals either from the CRB Xenopus in Rennes, NASCO in the US or the EXRC; only a few breed their own. I.e. every effort is made that specimens survive the transport in the best condition possible.
1.6b. How likely is the organism to survive existing management practices during passage along the pathway?	**likely**	**low**	No exhaustive information has been found. Movements of animals for their use in research activities is intentional, and as such there is no management practice in place to prevent the species being introduced into the risk assessment area. Specimens shipped to laboratories may be done mostly by specialised services, but the main reason seems to be the reduction of stress to which the animals will be subject to.
1.7b. How likely is the organism to enter the risk assessment area undetected?	**very likely**	**high**	The intentional introduction of animals in the risk assessment area for use in research activities cannot go undetected, but those animals being

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

			et al. 2012). Releases or escapes and successful establishment of populations in the wild have been attributed in the past to this pathway. Laboratory security has been increasingly improving over the years so escapes are now considered much less likely. However, people working with alien species (even when in special facilities) should be made aware of the issues concerning release (John Measey, pers. comm. 2018). In this regard, it should be noted that guidance available in the public domain usually refers to animal welfare considerations with little emphasis to biosecurity rules. Reed (2005) for example states: *"Containers must prevent escape and should allow space for sufficient volume and depth of water and for enrichment such as refuges to be added. A tank size of 65 x 45 x 45cms has been recommended ... for holding four to five frogs in 5 to 10 litres of water. A tank of this depth should (depending upon the water depth) prevent Xenopus from jumping out. An overhanging lip will further act to prevent escapes"*. Similarly, the UK Code of Practice for the Housing and Care of animals bred for scientific purposes (UK Home Office, 2014) is mostly about animal welfare and not about prevention of environmental impacts due to escapes or releases of animals in the environment. In conclusion, the likelihood of the species being intentionally released in the wild, in a suitable habitat, cannot be excluded. Furthermore, there is

			always the risk of unexpected events which may cause the escape of the animals, as was the case in Portugal where a laboratory experienced flooding, although this event was not confirmed (Measey et al. 2012, Sousa et al. 2018). As an additional remark, Measey et al. (2012) expressed concern about the future risk of laboratory populations of *X. laevis* which - due to the possible replacement of this species with *X. tropicalis* as a research model organism - may be dismissed and dumped in the environment. In fact, although there are no documented instances with respect to academic replacement of model organisms, there are examples of this in the US where school pets became illegal to keep and animals were dumped (John Measey, pers. comm. 2018). Given the instruction to euthanize a large number of animals, many people will still choose to dump living animals into the natural environment, if they do not have specific knowledge of the potential consequences of such release (John Measey, pers. comm. 2018).
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**likely**	**high**	In current conditions, it is likely that the species can still enter into the wild in the risk assessment area based on this pathway. The species is already present in research facilities in Europe, and has already been recorded in the wild in the risk assessment region, possibly as a consequence of this pathway.

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

			that invasive populations of *X. laevis* are established well beyond the species' multivariate realized niche in southern Africa (Rödder et al. 2017). The maps shown in the paper by Ihlow et al. (2016) and Rödder et al. (2017) do not allow a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. A recent study by Ginal et al. (2020) revealed a much higher risk of invasion, especially for most parts of Europe. However, it does not provide detailed maps for countries and biogeographic regions showing the precise potential invasive range of the species in Europe. In conclusion, the likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change condition is likely to be the same as in current conditions (see above). However, no documented evidence exists to support this statement.

PROBABILITY OF ESTABLISHMENT			
Important instructions: • For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established. If the species is established in all Member States, continue with Question 1.16.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	**very likely**	**high**	According to Measey et al. (2012) a large suitable climatic potential was identified for most of southern Portugal and adjoining Spain, as well as central and southern France, and mainland Italy. Such data are consistent with the findings of Ihlow et al. (2016), who highlighted areas in the main Mediterranean islands (namely the Balearic Islands, Sardinia, and Corsica) to be highly vulnerable to potential invasions. According to Measey et al. (2012), a few suitable areas were found in the United Kingdom outside southern coastal areas, plus Greece, Ireland, Germany, Belgium, Denmark and the Netherlands (for details see maps developed by Measey at al. 2012). Ihlow et al. (2016) predict only moderate probability for Great Britain. Despite this, the species has had persistent populations in the UK that are now exinct.
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it	**very likely**	**high**	*X. laevis* is a vigorously adaptable species which may virtually inhabit any type of water bodies, including lakes and rivers, as well as

and the organism's current distribution?			permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides that, *X. laevis* thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005). These habitats are widespread all over the EU, including countries where the species is not yet established. Additionally, Moreira et al (2017) have recently documented that this species can breed in both lotic and lentic environments. The use of lotic habitats may open up even more habitats for breeding, and may contribute to the maintenance of the invasive population even in the absence of lentic sites.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	**widespread**	**high**	*Xenopus* species in sub-Saharan Africa inhabit virtually all water bodies, including large rivers and lakes, as well as permanent and temporary ponds over a wide range of altitudes and temperatures (Measey 1998). According to John Measey (pers. comm. 2018), animals are often found in very low abundance (and occasionally very high) in natural systems, but numbers can become overwhelming in modified habitats. The latter are normally enriched and eutrophic, which probably helps build up their numbers. Measey et al. (2012) pointed out that comparatively few reports exist of *X. laevis* in

			its natural habitat, hence the lack of knowledge about the native ecology and natural dispersal of this globally invasive species. However, there are a few studies on the invasive range which provide useful information on this regard. For example, according to a study on habitat suitability carried out in Chile, Lobos et al. (2013) confirm that lentic aquatic environments, with slow drainage and murky waters, highly connected, human-disturbed, and part of an irrigation system of small streams and canals, account for the highest probabilities of successful establishment of *X. laevis* within the area of invasion. As reported by Lobos & Jaksic (2005) *X. laevis* in Chile lives from almost sea level up to 620m, and inhabit quite a diverse array of habitats with regard to water temperature, dissolved oxygen, pH, and electric conductivity, indicating a high degree of adaptability and colonization potential. In France, the species is found in a wide range of aquatic habitats and seems to have progressed very quickly along river valleys. Large rivers do not present barriers to the expansion of the species (and may even support further spread). It is likely that it used for its spread the watercourse or the wetlands associated to the river Loire (LIFE CROAA, pers. comm. 2019). Also a recent study by Ginal et al. (2020) has pointed out the

			importance of river networks in assisting the expansion even in areas with moderately suitable conditions. In the case of the French population, the same authors expressed concern that an ongoing shift in the fundamental niche of the French population combined with scenarios of climate change could lead to further expansion into new ranges, which are currently predicted to be unsuitable for this species. In particular, the French invasive population seems to make use of hydrographic networks and has now reached the Loire River catchment which covers about 20% of the French national territory (Ginal et al. 2020). It is also worth noting that animals are found in isolated ponds that are unconnected, like in France and Sicily, which means that *X. laevis* is able to move overland (John Measey pers. comm. 2018). Overland movements amongst various types of lentic and lotic water bodies seem to be frequent in this species (Ginal et al. 2021) although the possible contribution of man-mediated releases should be also taken into account in these cases. A study by Moreira et al (2017) in Portugal documented that *X. laevis* breeds in small streams and ponds, suggesting that while lentic sites are most likely responsible for population booms, the potential reproduction

			in lotic sites may contribute to the maintenance of the invasive population even in the absence of lentic sites. There are numerous ponds, lakes and other water bodies that are potentially suitable for the survival, development and multiplication of this species in the risk assessment area.
1.16. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area ?	**NA**		There is no information suggesting that *X. laevis* requires another species for critical stages in its life cycle.
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	**very likely**	**high**	*X. laevis* has only a few competitors that may prevent its establishment in the EU, the most effective being fish (e.g. eels), but the species may find suitable habitats where such competitors are absent (see Tinsley et al. 2015a). According to John Measey (pers. comm. 2018) fish appear to influence where animals will colonise, and it is possible that this could be used to prevent further invasions (e.g. a ring of ponds with introduced fish). Other non-native competitors are mentioned by Prinsloo et al. (1981), i.e. the Chinese silver carp, *Hypophthalmichthys molitrix*, as both the tadpoles and the silver carp compete for phytoplankton as food, and the Chinese bighead carp *Aristichthys nobilis* (zooplankton feeder). *Xenopus laevis* may live in the same ponds as crabs and terrapins, and undergo

			significant predation and mutilation from these groups, but without moving (John Measey, pers. comm. 2018).
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	**very likely**	**high**	In Africa, *X. laevis* have evolved morphological, behavioural, and biochemical predator avoidance strategies, and in extralimital situations, for example in California, it is likely that predatory pressure is considerably reduced (for example by occupying sites lacking predatory fish), thus contributing to the success and spread of *X. laevis* (McCoid and Fritts, 1980). Despite the lack of dedicated studies on the issue, the situation in the EU may be similar (as demonstrated also by the successful spread of the species in some countries). However, a recent study by Kruger et al. (2019) shows anti-predator response of *X. laevis* to a Dytiscid beetle (*Dytiscus dimidiatus*), probably a generic response, and even to the invasive crayfish *Procambarus clarkii*. These responses were observed in naïve tadpoles suggesting that anti-predator behaviour are evolving in the invasive range, in France at least. In principle, *X. laevis* can be a prey for several species, including fish, snakes, birds and mammals. For example, in the UK, *X. laevis* may be eaten by fish and amphibian predators

			including herons, American mink (*Neovison vison*) and, possibly, grass snakes (*Natrix helvetica*). Eels are potential predators too (Tinsley et al. 2015a). During a study in western France, Eggert and Fouquet (2006) showed that predation by the polecat (*Mustela putorius*) was deemed the major adult mortality factor, together with (assumed) freezing. Predation by gulls has been observed on a site with high density in a wastewater treatment unit (LIFE CROAA, pers. comm. 2019). In its native range in South Africa, *X. laevis* is eaten by large fish, turtles, frogs, snakes, aquatic insects, and birds (Lafferty & Page, 1997). This list is actually far longer. Almost every predator eats the adults, crabs eat the eggs and larvae, and odonates and fish eat the tadpoles (John Measey, pers. comm. 2018). Similarly, in its introduced range outside the EU, i.e. in Chile, three bird species were observed to prey on *X. laevis*: Night heron (*Nycticorax nycticorax*), Kelp gull (*Larus dominicanus*) and Burrowing owl (*Speotyto cunicularia*) (Lobos & Jaksic (2005). In the USA *X. laevis* is preyed upon by Two-striped Garter Snakes (*Thamnophis hammondii*). Large fish, and the American Bullfrog, (*Lithobates catesbeianus*) are considered to be potential predators as well (Lafferty & Page, 1997). Additionally, according to Prinsloo et

			al. (1981), the largemouth bass (*Micropterus salmoides*) is a known "biological control" against *X. laevis*. A recent study by Lobos (2020) carried out in Chile through laboratory assays provided evidence of the vulnerability of *X. laevis* to the eastern mosquitofish (*Gambusia holbrooki*). The results seemed confirmed by the recent establishment of *G. holbrooki* in a site which resulted in a collapse in the reproduction and adult populations of *X. laevis*. *Gambusia holbrooki* is an invaisve alien species in Europe, but its impact on *X. laevis* was not yet studied in this region. Regarding parasites and pathogens, *X. laevis* carries a rather diverse parasite load, and does not seem to be particularly affected by any of them. In Portugal for example, this species was found to be infected by autochthonous parasites, probably as spillover from *Pelophylax perezi* (Rodrigues 2014). However, the parasite burden was not as high as in the species they co-exist with, or as high as in the habitats where it is native, which in fact could enable this species to dominate the streams where it was recently introduced.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	**very likely**	**medium**	There are no specific management practices in place in the EU which may prevent the organism from establishing wild populations (as demonstrated by the successful establishment of the species in some

			countries). Fish species released (e.g. for sport and angling) may predate on *X. laevis*, like the non-native largemouth bass (*Micropterus salmoides*) but this is not considered as a practice targeted to the management of *X. laevis* in the risk assessment area. Release of *Micropterus salmoides* as biological control agent of *Xenopus laevis* was tried in South Africa (Prinsloo et al. 1981). The species may anyway occupy sites lacking predatory fish.
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	**moderately likely**	**low**	Overall, the species is known to thrive in highly disturbed habitats. For example, in southern California this is considered a common factor in all established populations (McCoid and Fritts, 1980). Therefore, it is likely that management of water bodies facilitates the establishment of the species by contributing to the creation of suitable habitats. However, there are opposite views. For example, management of UK water bodies and connecting habitats tends to be more intense than in many parts of the natural range of *X. laevis* which would in fact be more likely to hinder, rather than aid, establishment (NNSS 2011). Certain methods of wastewater treatment seems to facilitate the establishment of the species. In France and South Africa, the

			species thrives in units for the treatment of domestic wastewater. These are a string of connected ponds with no specific treatment but with eutrophic waters and higher temperature than other water bodies. In the context of the ongoing LIFE project CROAA (2016-2022), such ponds were fenced and a single unit yielded several thousand captured individuals in less than a year (LIFE CROAA, pers. comm. 2019).
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	**likely**	**medium**	The species went likely extinct by natural means in the UK. However, eradication at a few sites (Measey et al. 2012) using a deliberate, targeted eradication campaign to eliminate the species fairly rapidly was considered a possibility, although follow-up surveys and control measures would be necessary (NNSS 2011). Small eradication campaigns were carried out successfully in the UK, Spain, and the USA (Measey et al. 2012) but in general this was only possible in small areas and at an early stage of invasion. The chances of success seem related more to the specificities of the water bodies affected (e.g. type, size, and overall network) rather than to the biological properties of the species. For example, care must be taken about when this is done as individuals are capable of surviving in the ground for many months (John Measey, pers.

			comm. 2018). Otherwise it is clear that the appropriate methodologies need to be identified carefully in relation to the species biological properties. For example, high concentrations of Rotenone failed to eradicate clawed frogs in Los Angeles County (St. Amant, 1975), because clawed frogs are air breathers (McCoid and Fritts, 1980). In France, a complete eradication of the species is considered impossible in the valley of the Loire due to the large size of the population and the high landscape connectivity of potential habitats (LIFE CROAA, pers. comm. 2019). Furthermore, survival of a handful of individuals could be enough to prevent complete eradication (a single gravid female can contain from 1,000 to 27,000 eggs per clutch, and will produce multiple clutches in a season under favourable conditions).
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	**very likely**	**high**	There are no specific studies providing an indication of the propagule pressure, but a single gravid female can contain from 1,000 to 27,000 eggs per clutch, and will produce multiple clutches in a season under favourable conditions (Global Invasive Species Database 2015), therefore in principle only a handful of individuals may be sufficient to start a new population. As shown by Lobos et al. (2014), the invasion

			of *X. laevis* in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population (although the number of released frogs is unknown, see Lobos and Jaksic 2005). According to Measey et al. (2012) propagule pressure plays a pivotal role in the establishment of *X. laevis*, as some populations became established after the release of large numbers of animals from breeding facilities (laboratory and pet supplies). Also De Villiers et al (2016) found that small numbers of *X. laevis* can produce hundreds of adults within relatively short periods (e.g. 18 months). *X. laevis* is principally aquatic throughout its life. In general, tadpoles take 3 months to metamorphosis, and sexual maturity is achieved within one year (Global Invasive Species Database 2015) although this may happen only in certain circumstances (i.e. this was in California and may even be greater than in its native range in South Africa according to John Measey, pers. comm. 2018). Field studies by Tinsley et al. (2015a) showed that in favourable conditions there may be good recruitment, fast individual growth rates and large body size; maximum longevity exceeds 23 years. After all, the reproductive biology of the species seems very flexible. For

			example in its alien range in the US *X. laevis* reproduction reportedly occurred in most months of the year, in contrast to the shorter breeding season in South Africa (McCoid and Fritts, 1980). Also in its native range in South Africa, where the breeding season is poorly reported, seems to cover two distinct areas where the breeding times are opposite (John Measey, pers. comm. 2018). In the UK, *X. laevis* appeared not to breed prolifically under current climatic conditions, but a large recruitment event was considered possible should suitable weather conditions occur for even one season within the period covered by the occurrence of this species in the wild (NNSS 2011). In France, adults move between neighbouring ponds (about 15% of marked individuals). This means that they can evade local unfavourable condition in a site to move to another. Their good dispersal capacity, physiological resistance, and high productivity seems to make populations rather resilient (LIFE CROAA, pers. comm. 2019).
1.23. How likely is the adaptability of the organism to facilitate its establishment?	**very likely**	**high**	As summarized by Measey et al. (2012) *X. laevis* is characterized by a suite of physiological and behavioural traits which makes this anuran very robust and versatile, enabling it to cope with dehydration, high levels of salinity, starvation and anoxic

			conditions. Both adults and larvae perform well over a wide range of temperatures, and larvae can metamorphose in a wide range of temperatures. Behavioural traits include their capability to migrate overland, to survive drought by burrowing into drying mud and to starve for up to 12 months (see Measey et al. 2012, Tinsley et al. 2015a). For example, during drought in the UK, *X. laevis* could survive in isolated pools in the river course, in subterranean water bodies and by burying themselves in mud (Tinsley et al. 2015a). Additionally, *X. laevis* shows specific adaptations to aquatic life, including retention of the lateral line system in adults, aquatic chemoreceptors and a body structure particularly adapted for swimming (Elepfandt 1996). *X. laevis* is a very adaptable species, which may virtually inhabit any type of waterbody, including lakes and rivers, as well as permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides *X. laevis* thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005). After all, as pointed out by Tinsley & McCoid (1996), the hardiness which has made *X. laevis*

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

			algal populations eaten by their tadpoles (Tinsley and McCoid 1996), although tadpoles are limited to a certain niche by being obligate suspension feeders (John Measey, pers. comm. 2018). A recent study by Kruger et al. (2019) shows anti-predator response of *X. laevis* to a Dytiscidae beetle (*Dysticus dimidiatus*), probably a generic response, and even to the invasive crayfish *Procambarus clarkii*. These responses were observed in naïve tadpoles suggesting that anti-predator behaviour are evolving in the invasive range, in France at least.
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	**likely**	**high**	As shown by a study by Lobos et al. (2014), the invasion of *X. laevis* in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population (although such low diversity may be not as meaningful as claimed as the study focused on mtDNA, according to John Measey, pers. comm. 2018). Therefore, low genetic diversity is not expected to be a problem for the species invasion process. It is also worth mentioning that these are tetraploid animals, and that this may mitigate against potential bottlenecks (John Measey, pers. comm. 2018).

1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	**very likely**	**high**	The species has already shown to be able to successfully establish viable populations in the risk assessment area, e.g. in Portugal, France and Italy.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	**likely**	**medium**	It is likely that high number of individuals are still kept and bred in captivity in the risk assessment area, which leads to a certain risk of some being intentionally or accidentally released in the wild, building up casual occurrences (like happened in the past and led to the occurrence of the populations recorded in the risk assessment area and beyond).
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	**very likely**	**high**	According to the studies carried out by Measey et al. (2012) and Ihlow et al. (2016) suitable areas (plus some limited optimal areas) fall within the Mediterranean and Atlantic biogeographic regions, as well as the Continental and Alpine regions. Established populations are already present in the former, but not in the latter. A recent study by Ginal et al. (2020) revealed a much higher risk of invasion, especially for most parts of Europe. However, it does not provide detailed maps for countries and biogeographic regions showing the precise potential invasive range of the species in Europe.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	**very likely**	**high**	Under foreseeable climate change, the global invasion potential of this species in 2070 assessed by Ihlow et al. (2016) following four

			IPCC scenarios (i.e. RCP2.6, RCP4.5, RCP6, RCP8.5) may expand into northwestern Europe and the Mediterranean area. The maps shown in the paper by Ihlow et al. (2016) do not allow a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions may become suitable for the species. A recent study by Ginal et al. (2020) revealed a much higher risk of invasion, especially for most parts of Europe. However, it does not provide detailed maps for countries and biogeographic regions showing the precise potential invasive range of the species in Europe.

PROBABILITY OF SPREAD

Important notes:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)	**moderate**	**high**	As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and rivers as migration corridors, but also performs terrestrial migrations (even without rainfall). It should be noted, however, that movement through streams and irrigation channels appears to be much faster than overland movement (Fouquet and Measey 2006). In a study that compared *X. laevis* invading an urban area with 2 other species, Vimercati et al (2017) suggested that they may be slower, but build up densities much higher and are arduous to detect. Indicative figures of estimated rate of dispersal are available from a few studies in both the species native and alien range. For example, estimated annual spread of feral populations varied between 1 and over 2 km in France (Fouquet and Measey 2006, LIFE CROAA, pers. comm. 2019) and 5.4 km in Chile (Lobos & Jaksic 2005).

			In particular, a study by Fouquet and Measey (2006) in France showed that, while lotic corridors are used by this principally aquatic species, most ponds are colonised through overland migration. According to Fouquet and Measey (2006) *X. laevis* is able to detect the presence of non-colonised ponds at a distance, and orient towards them. According to Fouquet and Measey (2006) the terrestrial spread can be estimated at approximately 1 km per year. In Italy, although the rate of spread is not sufficiently assessed, observations were made of newly colonized ponds at a distance of between 400 and 700 m from the nearest pond occupied by *X. laevis*, where it is likely that most individuals disperse overland (irrigation ditches are not present in the area and the ponds are not connected with each other) or are facilitated by a few temporary streams (Measey et al. 2012). In France, the network of road ditches is dense and these can be filled with water during some periods of the year, which may contribute to enhance survival during overland migration (LIFE CROAA, pers. comm. 2019). Natural spread in the UK appears to have been very slow or non-existent (NNSS 2011) but animals occurred in a very particular system, and spread out of this area would have been very difficult (John Measey, pers. comm. 2018). Additionally, Measey and Tinsley (1998)

			reported a female travelling 0.2 km in 48 hours. Also in Portugal, according to the result of a recent study by Ginal et al. (2021) the spread of the *X. laevis* population was comparatively slow compared to the populations in Sicily and France which seem to be spreading fast. This was likely due to the complex topology affecting the habitat connectivity, along with a generalised poor habitat quality of the streams and/or presence of predatory fish, as well as some inherent features of the Mediterranean climate. Overland dispersal rates appear to be slower, compared to situations with ponds close to downstream dispersal corridors (Measey et al. 2012), but as reported by both Faraone et al. (2008) and Fouquet and Measey (2006), population dispersal by terrestrial movement seems prevalent in Italy and France. In particular, in France overland movements of 0.5 km per year are reported (Grosselet et al. 2006), and an adult female followed by radio telemetry moved overland 80 m from a pond through a pasture, crossing a wooded hedge and reaching a puddle 20 centimetres deep (Eggert and Fouquet 2006). According to Measey (2016) distances moved overland were from 40 m to 2 km (although the 2 km distance could have included use of a river), which is comparable to distances travelled by other terrestrial amphibians. There is no apparent difference between native and invasive ranges, besides, walls and thick vegetation are regularly

			traversed). In fact, in native populations in South Africa a female moved over 2.3 km in less than 6 weeks (De Villiers and Measey 2017). Louppe et al. (2017) found differences in mobility at the range edge of an expanding invasive population of *X. laevis* in the west of France; in particular individuals from the range edge had a greater stamina and had longer legs compared to individuals at the centre of the range, suggesting fast evolutionary optimization of dispersal abilities. This of course may have implications for conservation because spatial sorting on the range edge resulting in the evolution of locomotory capacity may lead to an accelerated increase in the spread of this invasive species in France. Also, Courant et al. (2017b) found that the level of resources allocated to reproduction was lower at the periphery of the colonized range compared to the centre and may be the result of changes in trade-offs between life-history traits. Such a pattern could be explained by interspecific competition or enhanced investment in dispersal capacity. There is evidence that additional translocations by humans within the risk assessment area may occur, as has been the case in Chile (Measey, pers. comm), however intentional anthropogenic “spread” via release or escape was dealt with in the introduction and entry section (see guidance in the heading above), to avoid duplication of

			information regarding the relevant pathways.
2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	**minimal**	**low**	There is no evidence of spread by human assistance in the risk assessment area, with the exception of intentional releases or escapes from captive bred populations, which however pertains to the mechanism of entry (hence this is discussed in the relative section). For example, a new Sicilian population of this species was recently described by Faraone et al. (2017) according to whom the hypothesis of natural expansion along the river basin is doubtful, while the occurrence of a man-mediated introduction event is plausible (although it is not clear whether it could originate from individuals caught in the wild or from labs). Therefore this is to be considered in the context of new entries. On this regard, Lobos & Jaksic (2005) pointed out that all calculations of spread rate should be taken with caution given the possibility that there have been additional translocations by humans. No detailed information was found on the potential transport of *X. laevis* adults and tadpoles with fish lots, as documented for other amphibian species (e.g. the American bullfrog *Lithobates catesbeianus*), but there is at least one case in France of suspected translocation of individuals with fish restocking (LIFE CROAA, pers. comm. 2019).

			Sediment transport from waste water units also includes potential possibilities to transport frogs over larger distances (LIFE CROAA, pers. comm. 2019).
2.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 2.3 to 2.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	1) Corridors (Interconnected waterways / basins / seas). 2) "Unaided (Natural dispersal across borders of invasive alien species that have been introduced)".		The following pathway is involved in the spread of the species: 1. Corridors (Interconnected waterways / basins / seas). This pathway fully overlaps with "Unaided (Natural dispersal across borders of invasive alien species that have been introduced)". The main difference between the two pathways is that in the first one the species will move through the man-made infrastructures occurring in the area (i.e. interconnected waterway corridors such as channels, ditches, etc.) serving as Corridors with its own capabilities. Otherwise, in the Unaided category, the species is expected to move without any support from either humans or infrastructures. For example, the species is able to spread through overland movements (see details on point 2.1 above) which by the way are intrinsically part of the movements through the waterway corridors. For this reason both pathways have been covered in the risk assessment under one single heading (Corridors (Interconnected waterways / basins / seas). The likelihood of spread in the Union based on these pathways is very high, since the likelihood

			of survival and reaching a suitable habitat is also very high, as documented above. There is evidence of mass overland movements of animals, estimated to number several thousand (e.g. when water bodies dry-out), and that may be driven, at least in part, by the existence of populations with high densities (Measey 2016).
Pathway name:	**[Corridors (Interconnected waterways / basins / seas)]** including **[Unaided (Natural dispersal across borders of invasive alien species that have been introduced)].**		
2.3a. Is spread along this pathway intentional (e.g. the organism i released at distant localities) or unintentional (the organism i a contaminant of imported goods)?	**unintentional**	**high**	
2.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?	**likely**	**high**	As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and rivers as migration corridors. There are no specific studies providing an indication of the propagule pressure, but the species is known to have used this pathway successfully in the risk assessment area. However, the likelihood of this pathway to contribute effectively to the species spread is also related to the overall suitability of the area colonised. In the UK for example, small-scale migration was recorded but overall *X. laevis* did not show any evidence of dispersal into apparently favourable ponds connected by

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

2.6a. How likely is the organism to survive existing management practices during spread?	**likely**	**medium**	No relevant management practices exist which may prevent the natural spread of the species in Europe. On the contrary, there may be practices that may favour the spread of the species. For example in Chile, the common practice of emptying dams once a year (to extract silt) or the extraction and transport of sediments from wastewater units in France may aggravate the situation by forcing the animals to migrate off periodically (Lobos & Jaksic 2005; LIFE CROAA, pers. comm. 2019).
2.7a. How likely is the organism to spread in the risk assessment area undetected?	**very likely**	**high**	The detection of single individuals or even new populations can be difficult, given the aquatic and elusive nature of the species. Several introduced populations of *X. laevis* have gone undetected for long time periods, 2–25 years in some cases (Measey et al. 2012). Van Sittert and Measey (2016) estimated that invasion debt rates -— lag between the export of African clawed frogs and a rise in invasive populations -— were around 15 years.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	**very likely**	**high**	According to Measey et al. (2012) irrigation channels and streams or rivers appear to be the major routes for dispersal for many invasions When these run close to artificial dams or ponds, large populations quickly become established.
2.9a. Estimate the overall potential for spread within the Union based on this pathway?	**likely**	**high**	As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and

			rivers as migration corridors. Spread may depend on the presence of canals. For example in Chile, a rapidly expanding viniculture industry has been assumed to have the potential to aid the spread of this invader, through extensive irrigation corridors, into new and previously uncolonized areas (Lobos et al. 2013). However, the likelihood of this pathway to contribute to the species spread is also related to the overall suitability of the area colonised. In the UK for example, *X. laevis* has been unable to spread far by natural means, despite being established at a small number of sites in the UK for several decades. However, it must be considered that animals occurred in a very particular system, and spread out of this area would have been very difficult (John Measey, pers. comm. 2018). Habitat connectivity is poor in the UK and, in any event, it is rarely simultaneously warm and rainy enough to encourage long distance overland movements by this species (NNSS 2011). In France, to the contrary, the high density of water bodies produces a highly connected landscape for the species. Connectivity, however, also depends on land use and according to experiments, the ability to move varies on different surfaces: individuals have more problems progressing across grass cover than

			across an area with bare soil or forest litter (Vimercati et al. Manuscript in prep.). Sousa et al. (2018) did speculate that artificial lakes of a golf course built between two sites of occurrence of the species in Portugal may have facilitated the dispersal of the species, although this is not confirmed by any definitive evidence.

End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	**very difficult**	**low**	Effective containment measures to prevent the spread of *X. laevis* through the pathway above are the same as those to control/eradicate the species, hence their applicability is clearly context dependent, and depends on the size of the population and the invasion stage.
2.11. Estimate the overall potential for spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	**rapidly**	**medium**	According to the studies carried out by Measey et al. (2012) and Ihlow et al. (2016) suitable areas (plus some limited optimal areas) fall within the Mediterranean and Atlantic biogeographic regions as well as the Continental and Alpine regions. Established populations are already present in the former, but not in the latter.
2.12. Estimate the overall potential for spread in relevant biogeographical regions in foreseeable climate change conditions	**rapidly**	**medium**	Further warming of the climate due to climate change may benefit the species in colonising new areas through natural dispersal. For example, by the 2070s, climate change is predicted to increase suitability in the risk assessment area, although the maps shown in the paper by Ihlow et al.

			(2016) do not allow for a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions may become suitable for the species.

MAGNITUDE OF IMPACT

Important instructions:

- Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?	**moderate**	**medium**	*X. laevis* is as a generalist predator able to modify its diet according to available resources (Courant et al. 2017a). Evidence exists of the negative impact on local populations of amphibians, fish, and invertebrates (Measey et al. 2012). In fact *X. laevis* is known to predate on and compete with native amphibians, including eggs and larvae (Measey et al 2015), and is thought to be a cause of trophic cascades by the consumption of benthic macroinvertebrates (Measey, 1998a; Lobos & Measey, 2002). In contrast, *Xenopus* tadpoles are primarily phytoplankton feeders (Schramm 1986). In particular, in its native range, competition and

			predation toward other pipid frogs (not present in the EU) was reported -– i.e. on the IUCN Endangered Cape platanna (*X. gilli)* -– along with predation on other anurans -– i.e. the common Cape River Frog (*Amietia fuscigula)*, the clicking stream frog (*Strongylopus grayii* and the Southern Dainty Frog (*Cacosternum australis*) suggesting a high proportion of anurophagy, of either eggs, tadpole or adults (Vogt et al. 2017). In central Chile, *X. laevis* preys on essentially three major food types: insects, molluscs and crustaceans, while the only vertebrates found in local diets are *Xenopus* larvae (Lobos & Jaksic 2005). Indeed, predation on amphibians (including on *X. laevis* itself) represented the most frequent vertebrate taxon in several studies on the species diet (Measey et al., 2016). Another study by Vogt et al. (2017) found *X. laevis* to consume large quantities of anuran eggs and larvae. Lastly, fish, like the endangered tidewater goby *(Eucyclogobius newberryi)*, western mosquitofish *(Gambusia affinis)*, and Arroyo Chubs (*Gila orcuttii)*, were found in the gut contents of *X. laevis* inhabiting the estuary of the Santa Clara River, in California (Lafferty & Page, 1997). Given the lack of evidence of long-term irreversible ecosystem change, the impact was considered moderate (see Annex II and remark in point A3 above).

2.14. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?	**moderate**	**medium**	Overall, the species impact on the risk assessment area is similar to the impact described in regions beyond the EU. For example, according to Measey (1998) *X. laevis* in South Wales ate a wide variety and size range of prey. Zoobenthos and zooplankton made the largest contribution to diets, followed by terrestrial invertebrates. Vertebrate preys (other than eggs and larvae of the same species) were also present, i.e. a bank vole (*Clethrionomys glareolus*) eaten alive or recently dead, and a chick (unidentified) probably eaten as a carrion. In fact, it is important to consider that *X. laevis* is also able to detect and feed on carrion (Measey 1998). Amaral & Rebelo (2012) confirmed the predation by *X. laevis* on eggs and adults of native amphibians, as well as on native fish in Portugal. The diet included benthic preys, with water snails (Physidae) being the most important, followed by the invasive American crayfish (*Procambarus clarkii*), but also native amphibians (including *Rana perezi* skeletons and egg masses) and fish (among which *Cobitis paludica*, a vulnerable Iberian endemic). In Portugal, during an eradication program in Oeiras carried out within the LIFE project LIFE17/GIE/ES/000515 Invasaqua, a captured juvenile of *X. laevis* was reported to prey on *Iberochondrostoma lusitanicum* (https://www.wilder.pt/naturalistas/ra-de-unhas-africana-esta-invasora-e-um-caso-de-

			sucesso-em-portugal/), a critically endangered species according to IUCN red list (Crivelli 2006). In France, Grosselet et al. (2006) speculated that *X. laevis* may predate on eggs of large newts (i.e. *Triturus cristatus* and *Triturus marmoratus*). Also Courant et al. (2018a) showed that species richness of native amphibians was negatively related to the abundance of *X. laevis*, despite some methodological bias discussed by the authors themselves. In particular, in France a significant decrease in the proportion of nektonic macroinvertebrates was reported in ponds occupied by *X. laevis* (Courant et al. 2018b). A study by Faraone et al. (2008) in Sicily shows that the most important prey categories are nektonic and planktonic organisms, and confirmed the presence of *X. laevis* eggs and larvae as well as terrestrial invertebrates (odonates and mayflies) in the diet. Additionally, Lillo et al. (2010) showed that presence of *X. laevis* in Sicily is associated with a decline in the reproduction of native amphibians (namely *Discoglossus pictus*, *Hyla intermedia* and *Pelophylax synklepton esculentus*). However, no one native amphibian was present in the diet of the species. Only conspecific tadpoles were found, confirming the significant cannibalistic behaviour of this species. The study by Lillo et al. (2010) also shows that the almost total absence of overlap of the trophic niche suggests the lack of competition for trophic

			resources between the alien species and the native ones.
2.15. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?	**moderate**	**low**	In case of a future expansion of the species range, other native species may be affected. While there is no documented evidence of the species being able to cause the extinction of any native one, it is likely that the level of risk will at least be confirmed as "Moderate" also in the future.
2.16. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?	**moderate**	**low**	In the light of the suggested impact on the amphibian species occurring in Italy and France and protected by the Habitats directive (*Triturus cristatus* is listed in Annex II and IV, while *Triturus marmoratus* and *Discoglossus pictus* are listed in Annex IV), the decline in conservation value caused by *X. laevis* is considered as "Moderate".
2.17. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?	**moderate**	**low**	In case of a future expansion of the species range, other native species may be affected. While there is no documented evidence of the species causing the extinction of any native species, it is likely that the level of risk will at least be "Moderate" in the future.
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	**moderate**	**high**	The impact of *X. laevis* on ecosystem services is caused by predation with possible accumulative effects in the ecosystem, including increased competition with other species for food (see point 2.13 and 2.14) and its functioning as a pathogen

			vector (see point 2.28). *X. laevis* might also have indirect impacts on aquatic systems such as increasing water turbidity and nutrient release caused by *X. laevis* disturbing the sediment (Lobos and Measey 2002). Consequently, *X. laevis* has been reported to negatively affect the invaded ecosystems, and as a consequence has been ranked as having the second greatest impact on native ecosystems by any amphibian (Measey et al., 2016). See also the assessments by Kumschick et al. (2017a), Kumschick et al. (2017b) and Kraus (2015) already discussed in point A3 of this document.
2.19. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?	**moderate**	**high**	Overall, the species impact in the risk assessment area is likely to be similar to the impact in regions beyond the EU, as described above, namely on: 1) Provisioning (Biomass: Reared aquatic animals); 2) Regulation & Maintenance (Regulation of physical, chemical, biological conditions: Lifecycle maintenance, habitat and gene pool protection, Pest and disease control, Water conditions).
2.20. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?	**moderate**	**low**	There is no documented evidence of the species being able to cause other types of impact (compared to those reported in current conditions), hence the level of risk can be expected to be “moderate” in the future.

Economic impacts			
2.21. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management	**minor**	**low**	Due to increased predation and/or competition for food, *X. laevis* is known to interfere with aquaculture, leading to possible economic costs. While no quantitative estimates about the economic impacts are available, in South Africa, for example, *X. laevis* is considered a threat to fresh-water aquaculture of common carp (*Cyprinus carpio*) and Chinese silver carp (*Hypophthalmichthys molitrix*) mostly because of competition for food (Schramm 1987). Additionally, it is considered a constraint on the production of the giant freshwater prawn (*Macrobrachium rosenbergii*), mainly due to predation (Taylor et al., 1992). Outside its native range, in Japan, the African clawed frog was found to have an impact on aquaculture by preying on juvenile carp (Kokuryo, 2009). In particular, a study by Schramm (1987) in South African aquaculture ponds, revealed that farmed fish larvae constituted a large proportion of *X. laevis* stomach contents (up to 25%), and that small fish <1 g are particularly vulnerable (although it does not necessarily represent the typical diet of native populations, see Courant et al. 2017a). Furthermore, in a study by Schramm (1987), it seemed likely that competition with *X. laevis* tadpoles was at least partly responsible for the slower growth of *H. molitrix*. In addition to the above, a reported problem in

			South Africa concerns the mass migrations leading to large numbers of clawed frogs invading houses and clogging up irrigation pipes (Somma 2018, Tinsley et al., 1996), but also in this case no figure is available. Following the SEICAT scheme developed by Bacher et al. (2018), the impact category for this species should therefore fall in between Minimal concern and Minor. No information/data is available on the costs for management, despite the many management activities carried out on the species. The only exception is an estimation of the man days reported for the control of the species in a pond in South Africa (De Villiers et al 2016). According to the authors, 27 person days for 338 *X. laevis* from one impoundment were needed, while regular seining could require as little as eight person days per year. As a side note, according to John Measey (pers. comm. 2018) the impoundment size was 603 m^2 (see also Vogt et al 2017). Lastly, a LIFE project aimed at the control of *Xenopus laevis* - together with the American bullfrog (*Lithobates catesbeianus*) - is currently in progress (2016-2022) in France: LIFE15 NAT/FR/000864 LIFE CROAA - Control stRategies Of Alien invasive Amphibians in France (for details, see https://www.life-croaa.eu). The project, co-funded by the EU through the LIFE

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

			dispersal corridors (together with the eradication/control of *Lithobates catesbeianus* in several sites). The total budget of this project is of 3.43 million euro and it will be carried out in the period 2016-2022 by the Société Herpétologique de France and other partners. In particular, the costs sustained locally to fight against *X. laevis* in 2017 and 2018 amounted to 81.000 €. The activities were carried out on a small part of the main introduction site, with the aim of preserving a few sites considered to be at stake due to the presence of native species or located near dispersal areas (LIFE CROAA, pers. comm. 2019).
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	**major**	**low**	In case of a future expansion of the species range in the risk assessment area, the economic costs / losses associated with managing *X. laevis* may rise accordingly.
Social and human health impacts			
2.26. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).	**minimal**	**low**	No information has been found on the issue.
2.27. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.	**minimal**	**low**	No information has been found on the issue.
Other impacts			
2.28. How important is the impact of the organism as	**minor**	**low**	The most serious impact usually attributed to *X.*

food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?			*laevis* is related to its potential role in the introduction and spread of the chytrid fungus, *Batrachochytrium dendrobatidis (Bd)*, the cause of amphibian deaths and population declines in several parts of the world (Weldon et al. 2004). Bd disease has been implicated in mass mortalities and widespread declines in European amphibian species, like common midwife toad (*Alytes obstetricans*) (Bosch et al., 2001) and fire salamander (*Salamandra salamandra*) (Bosch & MartínezSolano, 2006) in Spain. However, to date there is no evidence that *X. laevis* has functioned in this role of Bd vector or has caused impact on native amphibians through this mechanism. For this reason, the impact of the species was considered "minor" by Kumschick et al. (2017b, see in particular the supporting information annexed to the relevant paper). As a remark, the same authors discussed a previous assessment by Kraus (2015) based on the assumption that *X. laevis* contributed to the spread of Bd which then caused declines in native species, but which is not demonstrated (De Busschere et al. 2016, John Measey, pers. comm. 2018). Hence a higher score would not be justified. *Xenopus laevis* was also identified as a potential vector of ranavirus (Robert et al., 2007). Although a causal link between *X. laevis* and the dispersal of these pathogens is not demonstrated (Measey et al. 2012), this frog could play a role in

			the spread of disease, by acting as an asymptomatical reservoir/vector for both diseases, without being susceptible or just suffering sublethal effects. This seems to be confirmed at least for the chytrid fungus by studies on either wild or captive animals in the UK, Chile, and USA (Tinsley et al. 2015b, Solís et al. 2010, Soto-Azat et al. 2016, Vredenburg et al. 2013), but not in France (Ouelletet al. 2012). Additionally, *X. laevis* may carry several other parasites and pathogens, like *Chlamydia* (Howerth et al. 1984, Reed et al. 2000) and many others, in both its native range and the alien range (Kuperman et al 2004, Tinsley, 1996). For example, according to Lafferty & Page (1997), three internal parasites were observed in or on the gut (although a complete parasitological assessment was not undertaken). The African tapeworm *Cephalochlamys namaquensis* was found in intensities from 6-25 individuals (including several mature adults) in the anterior duodenum. It was not previously reported outside of Africa, hence it may have entered other areas with this species. Ciliates of the genus *Nyctotherus* (0.25 mm trophs) were present in abundance posterior to the section of the gut where tapeworms occurred. Larval nematodes were encysted on the outside of the stomach wall (might be transferred to the birds that eat them, potentially leading to some pathology).

			A recent study (Schoeman et al. 2020) reports seven previously unrecorded nematode species (none of which could be identified to species level) parasitising *X. laevis* across South Africa. These are adult *Capillaria* sp. and *Falcaustra* sp. from the intestine, third stage larvae of *Contracaecum* sp. encysted in the body cavity, third stage larvae of *Paraquimperia* sp. and *Tanqua* sp. from the intestine and two different species of second stage nematode larvae from the lungs and kidneys, respectively. According to Schoeman et al. (2020) this result illustrates that *X. laevis* is an important parasite reservoir in its native range, with implications for its role in the invasive range. In France, only parasites originating from the frog's native range have been detected so far. This has been found in other areas suggesting that *Xenopus* also introduces its own parasites in the assessment area (Schoeman et al. 2019). Parasites of *X. laevis* on its native range have been detected at a high prevalence in western France (*Protopolystoma xenopodis*: prevalence 19 %, *Cephalochlamys namaquensis*: prevalence 63%). The same parasites have been found in distinct invasive *Xenopus* populations. There has been no attempt to determine whether these alien parasites colonized new amphibian hosts in Europe.
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	**NA**		No information has been found on the issue.

2.30. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?	**major**	**low**	The natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area, is not expected to mitigate the impact of *X. laevis* in relation to its role as a vector of dangerous parasites and pathogens to the native fauna.

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ANNEX I Scoring of Likelihoods of Events

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

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

ANNEX II Scoring of Magnitude of Impacts

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

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[18]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss	Widespread and irreversible effects on one /	Above 10,000,000 Euro	Long-term social change, significant loss of employment,

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

	or extinction, affecting several species with serious ecosystem effects	several services		migration from affected area. Widespread, severe, long-term, irreversible health effects.

ANNEX III Scoring of Confidence Levels

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

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

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

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

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

			organisms (competition, predations, spread of disease etc.)
	Genetic material from all biota	**Genetic material** from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to breed new strains or varieties; Individual genes extracted from higher and lower plants for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities *Example: negative impacts of non-native organisms due to interbreeding*
	Water[19]	**Surface water** used for nutrition, materials or energy	Surface water for drinking; Surface water used as a material (non-drinking purposes); Freshwater surface water, coastal and marine water used as an energy source *Example: loss of access to surface water due to spread of non-native organisms*
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for drinking; Ground water (and subsurface) used as a material (non-drinking purposes); Ground water (and subsurface) used as an energy source *Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.*
Regulation & Maintenance	**Transformation** of biochemical or physical inputs to ecosystems	**Mediation of wastes or toxic substances** of anthropogenic origin by living processes	Bio-remediation by micro-organisms, algae, plants, and animals; Filtration/sequestration/storage/accumulation by micro-organisms, algae, plants, and animals *Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics*
		Mediation of nuisances of anthropogenic origin	Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure) *Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.*
	Regulation of physical, chemical, biological conditions	**Baseline flows and extreme event** regulation	Control of erosion rates; Buffering and attenuation of mass movement; Hydrological cycle and water flow regulation (Including flood control, and coastal protection); Wind protection; Fire protection

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

			Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.
		Lifecycle maintenance, habitat and gene pool protection	Pollination (or 'gamete' dispersal in a marine context); Seed dispersal; Maintaining nursery populations and habitats (Including gene pool protection) *Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries*
		Pest and disease control	Pest control; Disease control *Example: changes caused by non-native organisms to the abundance and/or distribution of pests*
		Soil quality regulation	Weathering processes and their effect on soil quality; Decomposition and fixing processes and their effect on soil quality *Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality*
		Water conditions	Regulation of the chemical condition of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes *Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication*
		Atmospheric composition and conditions	Regulation of chemical composition of atmosphere and oceans; Regulation of temperature and humidity, including ventilation and transpiration *Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)*
Cultural	**Direct, in-situ and outdoor interactions** with living systems that depend on presence in the environmental setting	**Physical and experiential** interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through active or immersive interactions; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions *Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.*

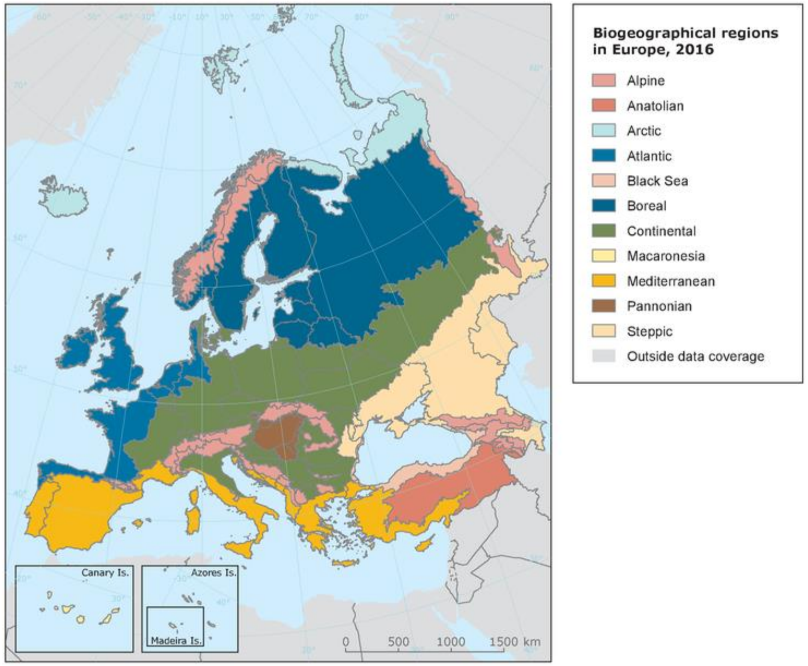
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable scientific investigation or the creation of traditional ecological knowledge; Characteristics of living systems that enable education and training; Characteristics of living systems that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

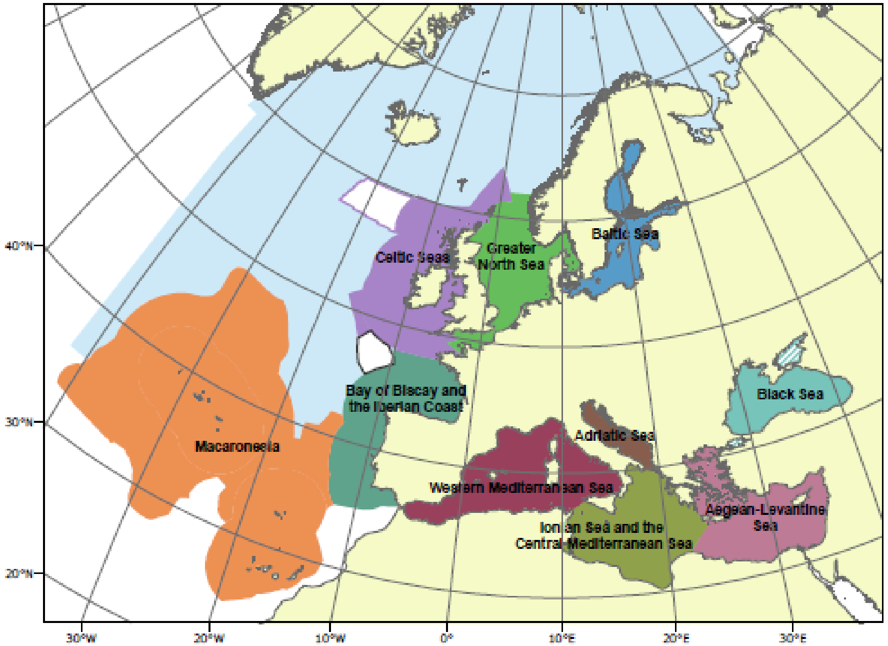
ANNEX V EU Biogeographic Regions and MSFD Subregions

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

and

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





ANNEX VI Species distribution model

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

The authors of both papers supplied shapefiles with the projected suitable regions, revealing marked differences in the European regions predicted to be suitable in the current climate (Figure 1). All models predict substantial suitable regions in Portugal, Spain, France and Italy. The models of Ihlow et al (2016) also predict large suitable regions in eastern Europe, based on a minimum training presence threshold. This threshold probably overestimates the suitable region in Europe as the species has been introduced and recorded in marginal conditions in northern Europe and suitability gradient maps shown in the paper suggest moderate to high suitability only in warm western Mediterranean regions. The predictions from the Measey et al (2012) model should be treated with caution as the Maxent model is less reliable than the ensemble model (John Measey pers. comm. 2018). Also, this study did not consider suitability under different emission scenarios (Table 1). Climate change projections supplied for the 2070s from Ihlow et al (2016) differed markedly between emissions scenarios and the Maxent and Ensemble models. However, these projections do not appear consistent across scenarios (e.g. RCP4.5 should be intermediate between RCP2.6 and RCP6.0, but it is not in all cases) and were probably influenced by an overly liberal minimum training threshold choice.

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

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

	Measey et al (2012)	**Ihlow et al 2016 (Maxent)**	**Ihlow et al 2016 (Ensemble)**
Number of native range records	1075	826	826
Number of non-native range records	124	99	99
Spatial resolution	2.5 arcminutes	2.5 arcminutes	2.5 arcminutes
Predictor variables from Worldclim	Isothermality (bio3) Minimum temperature of the coldest month (bio6) Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Precipitation seasonality (bio15) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of coldest quarter (bio19)	Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Mean temperature of the coldest quarter (bio11) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of the warmest quarter (bio18) Precipitation of coldest quarter (bio19)	Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Mean temperature of the coldest quarter (bio11) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of the warmest quarter (bio18) Precipitation of coldest quarter (bio19)
Modelling software	Maxent	Maxent	Biomod
Background definition	Radius of 250 km around the records	Radius of 250 km around the records	Radius of 250 km around the records
Reported predictor importance	Isothermality (27.4%) Minimum temperature of coldest month (19.8%) Precipitation of coldest quarter (11.7%) Mean temperature of warmest quarter (10.4%) Mean temperature of wettest quarter (8.8%) Temperature annual range (6.7%) Precipitation of wettest quarter (6.6%)	Precipitation of driest quarter (27.7%) Mean temperature of wettest quarter (16.8%) Mean temperature of coldest quarter (14.5%) Precipitation of warmest quarter (11.4%) Precipitation of coldest quarter (8.3%) Temperature annual range (7.0%) Mean temperature of driest quarter (6.2%) Precipitation of wettest quarter (6.2%) Mean temperature of warmest quarter (1.9%)	Mean temperature of coldest quarter (19.1%) Precipitation of warmest quarter (16.6%) Mean temperature of warmest quarter (13.9%) Precipitation of driest quarter (12.6%) Precipitation of coldest quarter (8.3%) Mean temperature of driest quarter (8.3%) Precipitation of wettest quarter (8.0%) Mean temperature of wettest quarter (7.6%) Temperature annual range (5.0%)

	Measey et al (2012)	**Ihlow et al 2016 (Maxent)**	**Ihlow et al 2016 (Ensemble)**
Threshold(s) to project suitable region	Minimum training presence and 10% training omission	Minimum training presence	Minimum training presence
Masking to prevent extrapolation	Multivariate Environmental Similarity Surface (MESS)	Multivariate Environmental Similarity Surface (MESS)	Variable clamping
Climate change scenarios	None	2070s under RCP2.6, RCP4.5, RCP6.0 and RCP8.5	2070s under RCP2.6, RCP4.5, RCP6.0 and RCP8.5

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

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

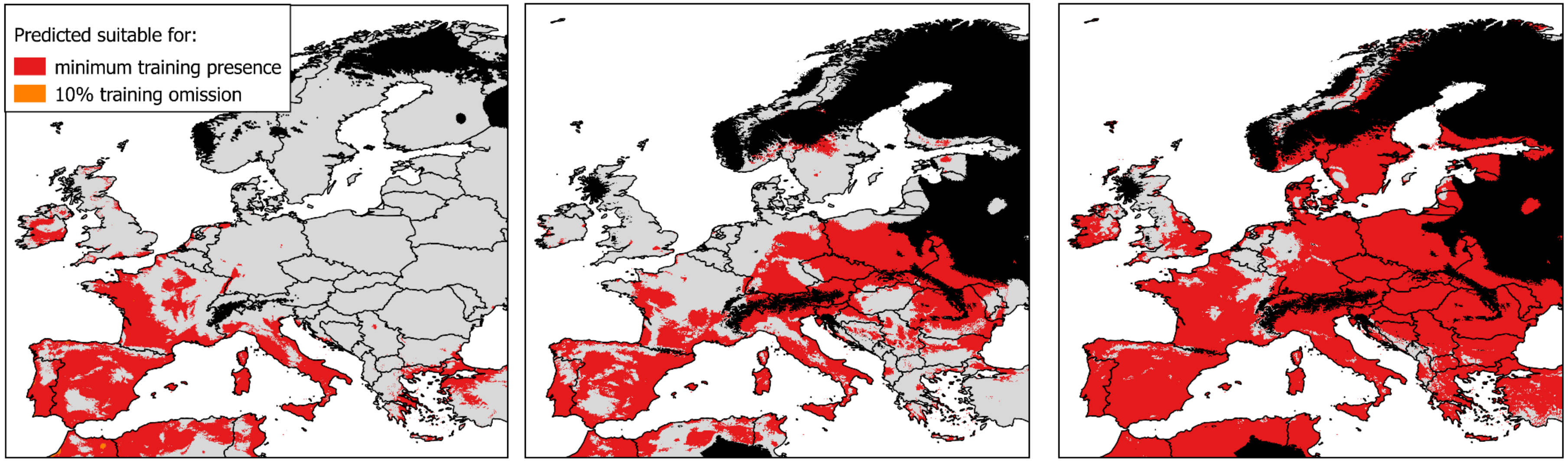
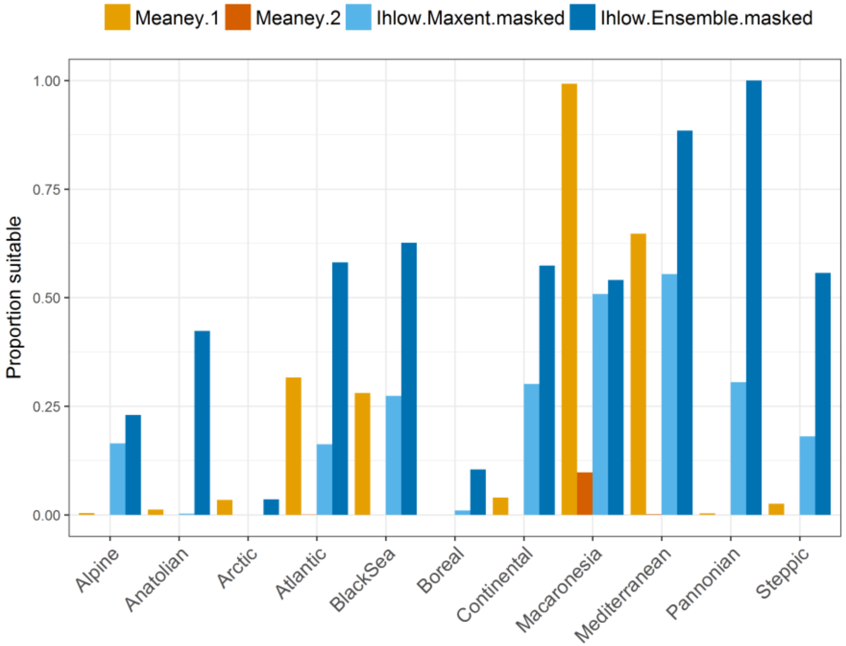


Figure 2. Variation in projected suitability among Biogeographical regions of Europe (Bundesamt fur Naturschutz (BfN), 2003) from the different model outputs supplied. The regions are shown in the right hand map. Measey.1 and Measey.2 differ based on thresholding by the minimum training presence or a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



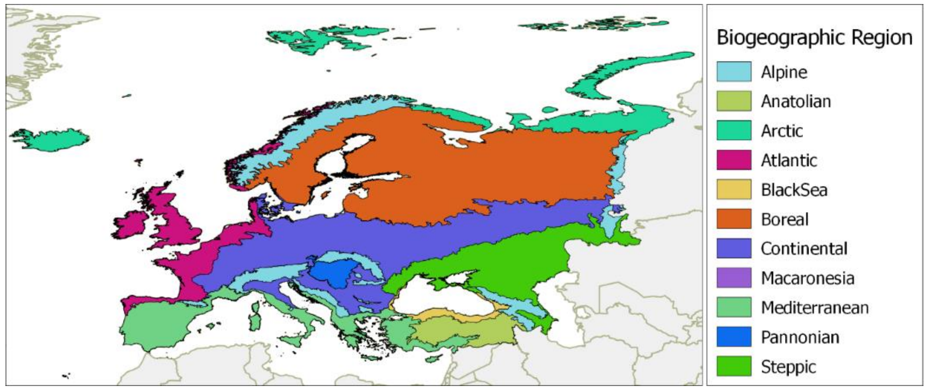
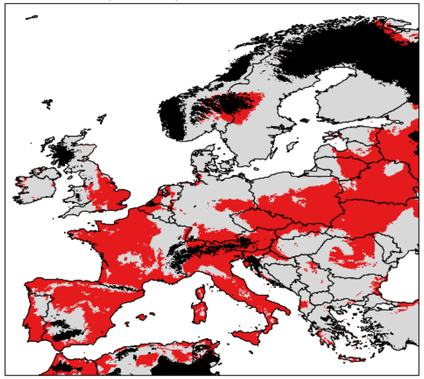
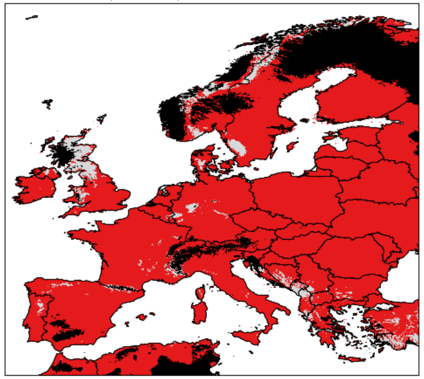


Figure 3. Projected European regions suitable for establishment by *Xenopus laevis* in 2070 under four emissions scenarios. Suitable areas above the suitability of the minimum training presence are shaded red. Regions where extrapolation prevented prediction are shown in black.

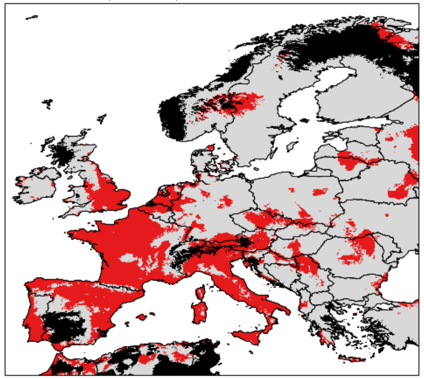
(a) Ihlow et al (2016) Maxent – RCP2.6



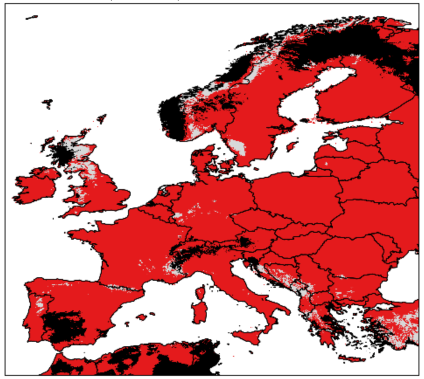
(b) Ihlow et al (2016) Ensemble – RCP2.6



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

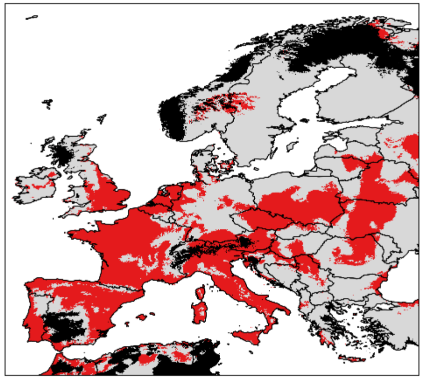


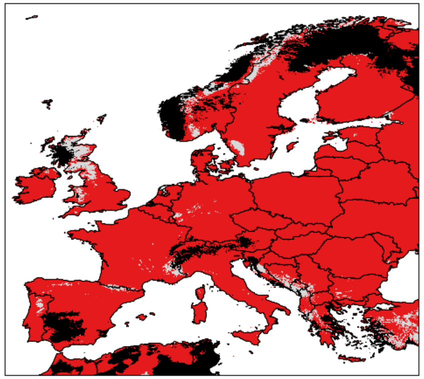
(d) Ihlow et al (2016) Ensemble – RCP4.5



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

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





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

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