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

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

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

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

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

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

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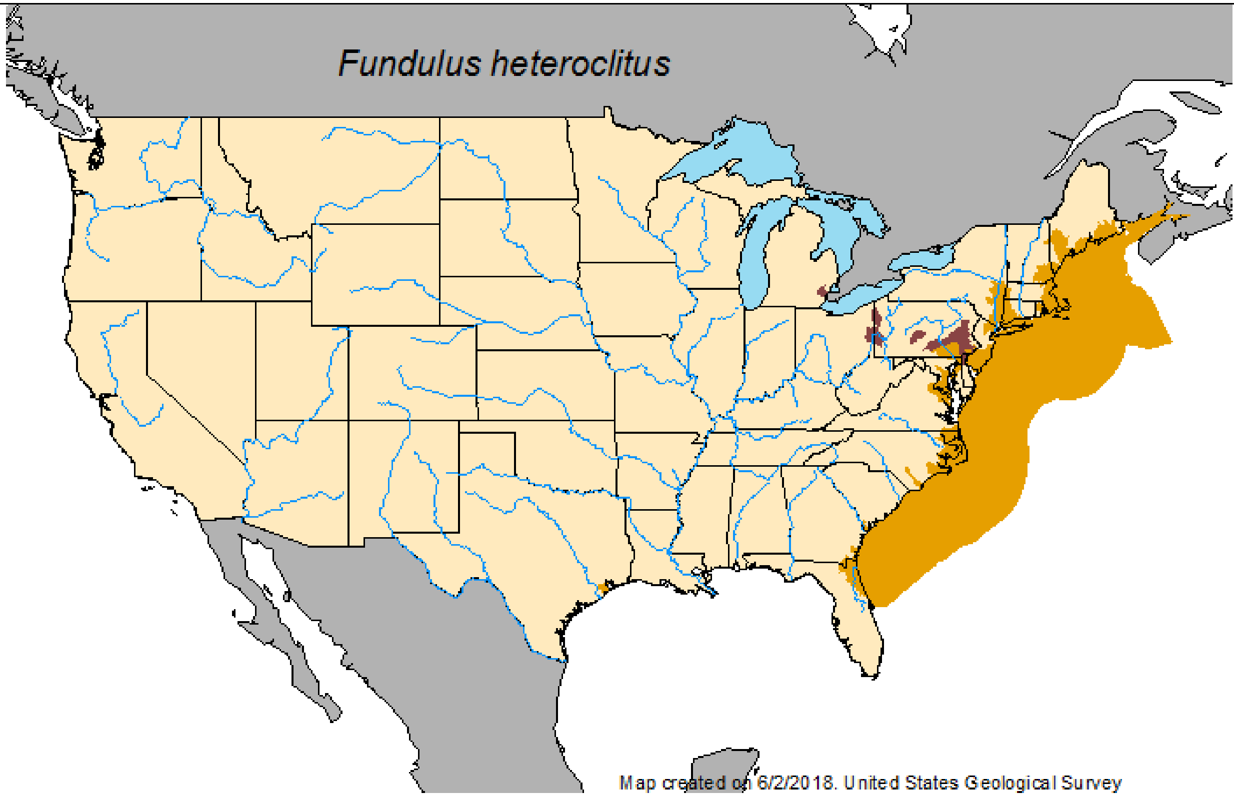
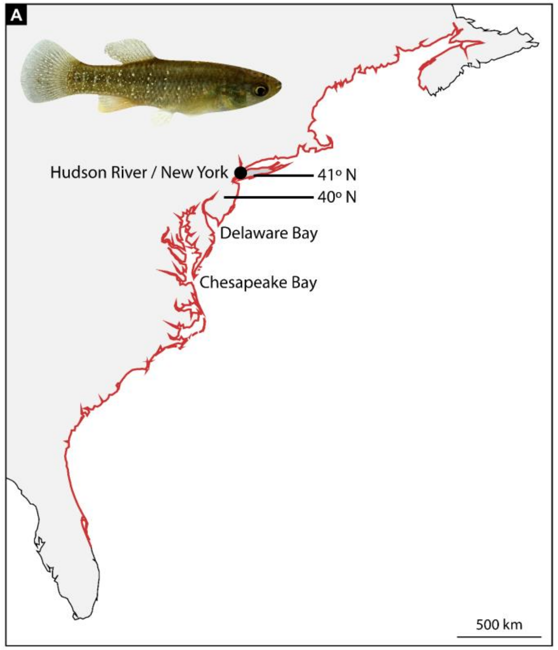
Contents

SECTION A – Organism Information and Screening

Organism Information	**RESPONSE**
A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Actinopterygii, Cyprinodontiformes, Fundulidae *Fundulus heteroclitus* (Linnaeus, 1766) Some frequent synonym names are: *Cobitis heteroclita* Linnaeus, 1766 *Valencia lozanoi* Gómez Caruana, Peiró Gómez & Sánchez Artal, 1984 *Fundulus heteroclitus heteroclitus* (Linnaeus, 1766) *Fundulus heteroclitus macrolepidotus* (Walbaum, 1792) Two subspecies have been traditionally recognized (*Fundulus heteroclitus heteroclitus* and *Fundulus heteroclitus macrolepidotus*) but they have an hybrid zone with clinal variation and are often considered not valid names nowadays (Relyea 1983; Page and Burr 2011; Froese & Pauly 2016; U.S. Fish and Wildlife Service 2017). Common names: mummichog; fúndulo (Spanish); fundulo, peixinho (Portuguese)
A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the wild, in confinement or associated with a pathway of introduction]	There are over 40 species of fundulids, all native to North America; Wiley & Ghedotti (2003) and Page & Burr (2011) provide taxonomic information to identify them. Parenti (1981) provides taxonomic keys to identify all cyprinodontiform genera. *Fundulus heteroclitus* is the only fundulid fish naturalised in the European Union, where there are about ten other cyprinondontiform fish present in the wild (see below). However, killifishes (a common term used in general for oviparous cyprinodontiforms) are popular in the aquarium hobby and many other species (including *Fundulus* spp.) are used in Europe. For instance, by October 2019, at least 5 *Fundulus* species (including "*Fundulus* s.p." (sic)) are listed as available from Spanish aquarium hobbyists (https://www.sekweb.org/censo/index.php?letra=f). Doadrio (2002) and Kottelat & Freyhof (2007) provide extensive information to distinguish *F. heteroclitus* from other similar fish. The only cyprinodontiforms native to the European Union are:

	Aphanius baeticus Doadrio, Carmona & Fernández-Delgado, 2006; *Aphanius fasciatus* (Valenciennes, 1821); *Aphanius iberus* (Valenciennes, 1846), *Valencia hispanica* (Valenciennes, 1846), *Valencia letourneuxi* (Sauvage, 1880), and *Valencia robertae* Freyhof, Kärst & Geiger, 2014. There are many other cyprinodontiforms endemic from parts of norther Africa, Turkey or the Middle East. The other cyprinodontiforms introduced to the European peninsula are poeciliids, which look considerably different: *Gambusia holbrooki* Girard, 1859, *Gambusia affinis* (Baird & Girard, 1853), *Poecilia reticulata Peters*, 1859, and *Xiphophorus maculatus* (Günther, 1866). All these species live in similar habitats as *Fundulus heteroclitus* and their ecology and life histories are similar. *Fundulus heteroclitus* was misidentified as *Valencia hispanica* and described as a new species (*Valencia lozanoi)* by Gómez, Peiró & Sánchez (1984) in the Iberian Peninsula, before it was realised that is was an introduced species (Fernández-Delgado *et al*., 1986; Morim, 2017). Therefore, *F. heteroclitus* could be misidentified with other species, namely other cyprinondontiforms.
A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)	An ecological risk screening of mummichog (*F. heteroclitus*) for the USA was performed by the U.S. Fish & Wildlife Service (U.S. Fish and Wildlife Service, 2017). In the Iberian Peninsula, where the species has been introduced, there are two published risk assessments (Clavero, 2011: Almeida *et al.*, 2013). Clavero (2011) focused mainly on the first stages of invasion (arrival and establishment) developing a specific procedure for the Iberian Peninsula. Almeida *et al.* (2013) applied the FISK approach (Fish Invasiveness Scoring Kit), obtaining an outcome of “moderately high” risk for the species. In Turkey, where the mummichog has not yet been introduced, a modified version of FISK, the AS-ISK (Aquatic Species Invasiveness Screening Kit), classified the mummichog as of medium risk (Tarkan *et al.*, 2017).
A4. Where is the organism native?	The native range of the species is the Western Atlantic region: from Gulf of St. Lawrence (Canada) to northeast Florida, USA (Froese & Pauly, 2016).

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

	 Figure A4b. Native distribution (red line) of *Fundulus heteroclitus* in the North American Atlantic coast. *F. heteroclitus* photograph from North American Native Fishes Association (2010). Figure from Morim (2017).
A5. What is the global non-native distribution of the organism outside the risk assessment area?	There are introductions within the United States such as New Hampshire (Scarola *et al.*, 1987) and western Pennsylvania (Trautman, 1981), possibly as a baitfish; some of these are failed introductions but it is established in the lower Susquehanna and Delaware drainages (U.S. Fish and Wildlife Service, 2017). FAO (2016) and FishBase (Froese & Pauly, 2016) list *F. heteroclitus* as introduced and established in Hawaii and The Philippines but the NAS database (Fuller, 2018), government webpages, or other sources do not list it as established or recently present in Hawaii (e.g. Englund, 2000, 2002) and The Philippines (e.g. Joshi, 2006; Cagauan, 2007).

<table>
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<td>A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established?</td>
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Figure A6. Known alien range (blue line and dot) of Fundulus heteroclitus in the Iberian Peninsula. Figure reproduced from Morim (2017).

Within Europe, Fundulus heteroclitus is only introduced and established in Spain and Portugal (see Fig A6), which falls within the ‘Mediterranean’ biogeographical region or “North-east Atlantic Ocean” and “Mediterranean Sea” marine regions (EEA, 2012).

Recorded: List regions
Freshwater / terrestrial biogeographic regions:
• Mediterranean.

Marine regions:
• North-east Atlantic Ocean, Mediterranean Sea.

Marine subregions:
• Bay of Biscay and the Iberian Coast, Western Mediterranean Sea.

Established: List regions
Freshwater / terrestrial biogeographic regions:
</td>
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	• Mediterranean. Marine regions: • North-east Atlantic Ocean, Mediterranean Sea. Marine subregions: • Bay of Biscay and the Iberian Coast, Western Mediterranean Sea.
A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change?	**Current climate:** Freshwater / terrestrial biogeographic regions: • Atlantic, Black Sea, Boreal, Continental, Mediterranean, Steppic Marine regions: • Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea Marine subregions: Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea. **Future climate:** Freshwater / terrestrial biogeographic regions: • Atlantic, Black Sea, Boreal, Continental, Mediterranean, Steppic Marine regions: • Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea Marine subregions: Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea,

	Aegean-Levantine Sea. *Fundulus heteroclitus* originally lives in brackish or salt water and secondarily nearby freshwater, and inhabits sheltered coastal areas such as saltmarshes, tidal creeks, estuaries or bays all year-round (Hardy Jr, 1978; Page & Burr, 2011) along the Atlantic coast of North America between Nova Scotia, Canada and Florida, USA. It withstands a wide range of salinities, from 0 to 120.3 ppm (Griffith, 1974), and temperatures, from -1.5 ºC (Umminger, 1972) to 36.3 ºC (Garside & Chin-Yuen-Kee, 1972), surviving abrupt changes in both parameters (Hardy Jr, 1978; Bulger, 1984). Its native range in eastern North America corresponds to the 'Cfa' and 'Dfb' Köppen-Geiger climate zone (Peel *et al.*, 2007), whereas much of central Europe is in the 'Cfb' zone (similar to 'Cfa'). In the Iberian Peninsula, it has established and spread in the 'Csa' zone. Therefore, it is likely to be able to establish in many European coastal areas in both current and future climates (Fig. A7 and A7b). However, it looks that its spread will be slow, given the lack of many introductions, the slow spread in the Iberian Peninsula, and its sedentary habits (see below). However, it has been recently suggested to be limited by the existence of benthic muddy saltmarsh environments, which are only found near major estuaries or lagoons areas (Morim et al. 2019). The effects of climate change in the progressive warming and salinity of estuaries water might favour its establishment and spread but should not change it much given its wide tolerance and native latitudinal range.

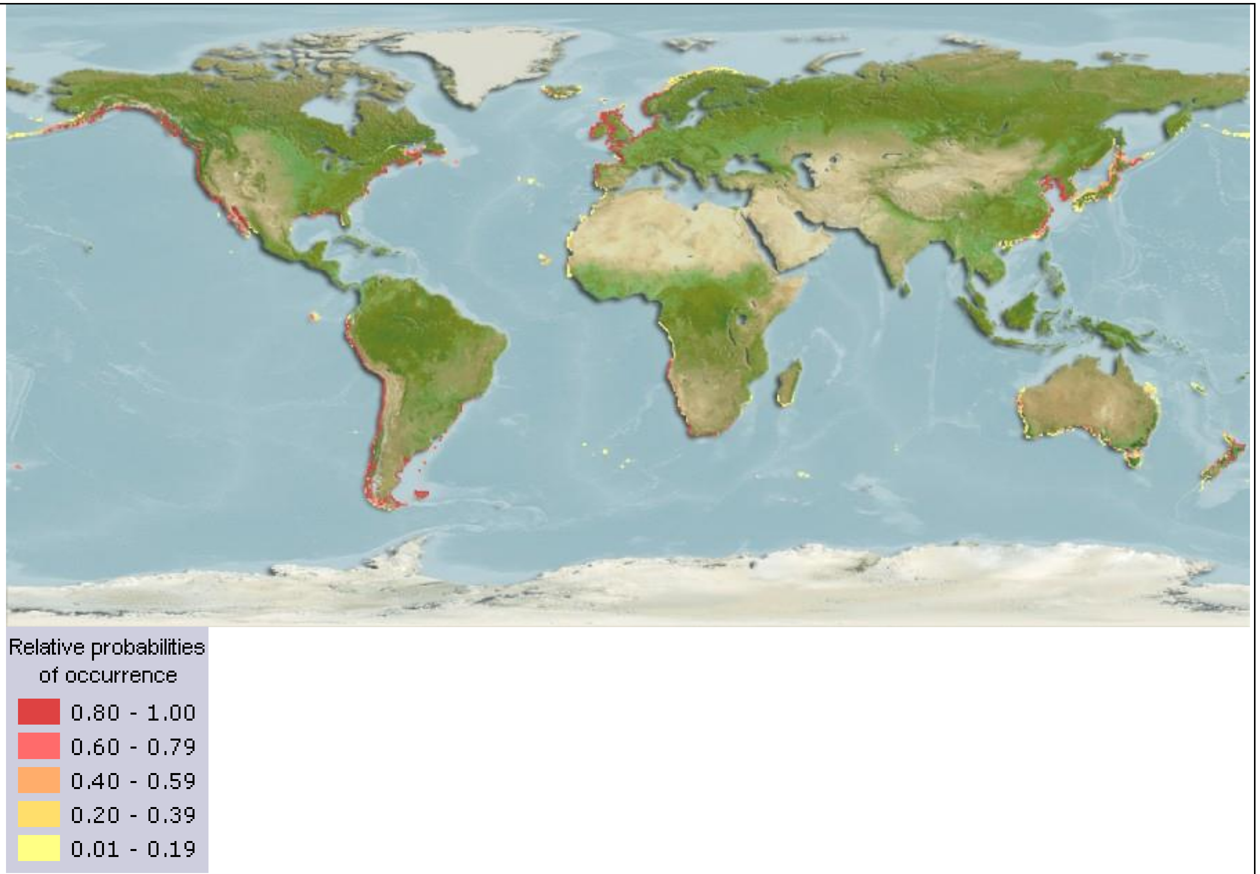
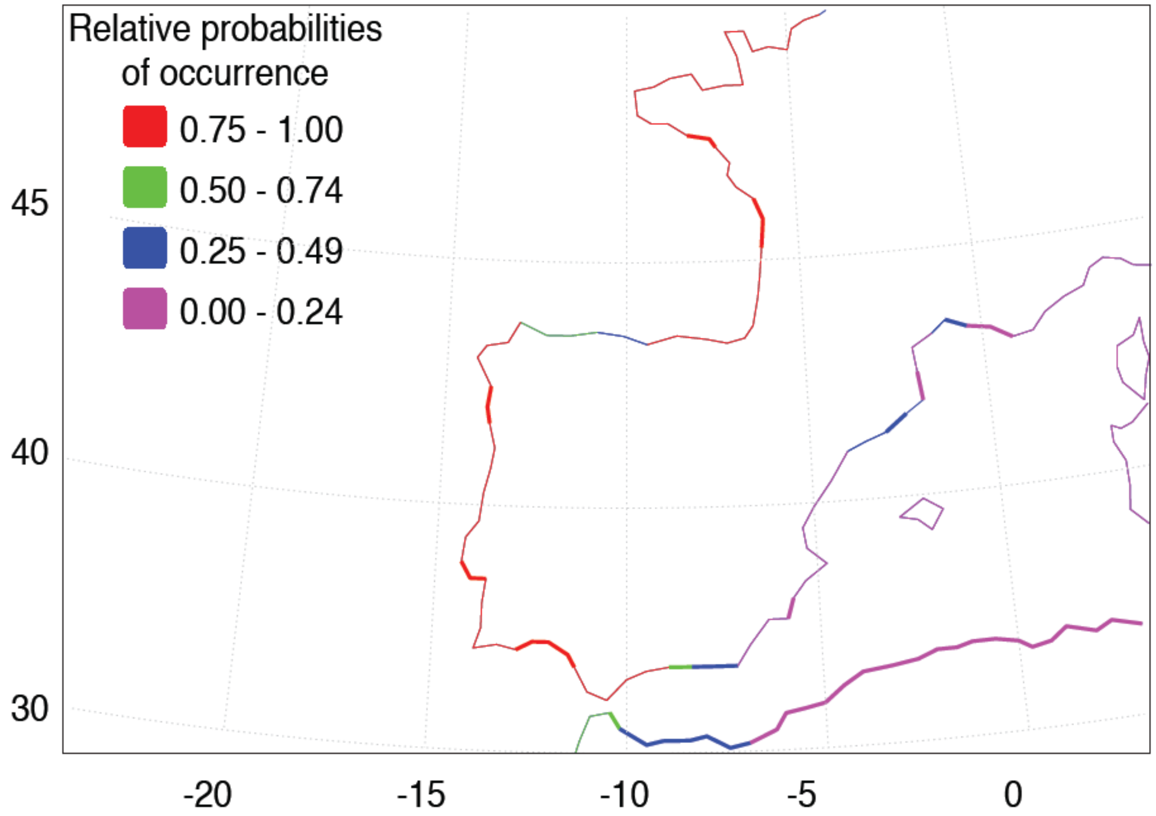
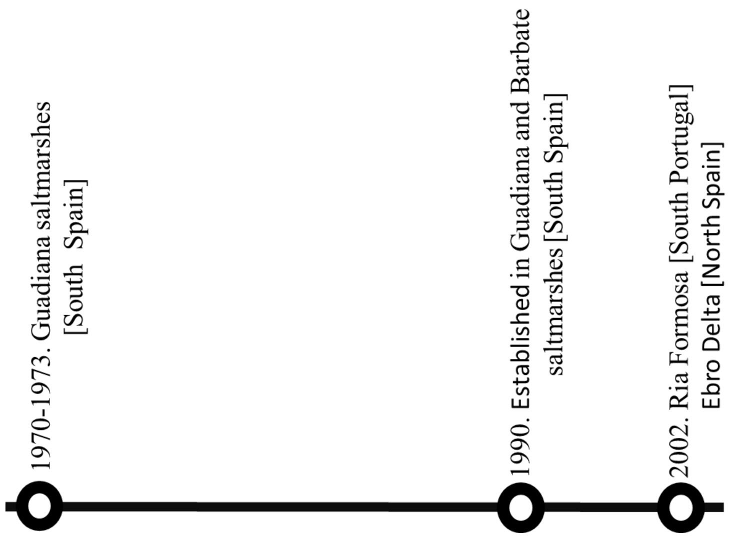


Figure A7. Computer generated potential distribution for *Fundulus heteroclitus* (Mummichog). www.aquamaps.org, version of August 2016. Web. Accessed 4 June 2018.

	 Figure A7b. Probability of occurrence of *Fundulus heteroclitus* in the western European and Mediterranean coastal environments, using AquaMap and environmental predictors, according to Morim et al. (2019; CC BY 4.0 Open Access). Those areas in bold show coastal seabed habitats with a mud content > 10%, where *F. heteroclitus* is very likely to establish, if introduced.
A8. In which EU member states has the species been recorded and in which EU member states has it established? List them with an indication of the timeline of observations.	Recorded: Portugal and Spain. Established: Portugal and Spain. Morim (2017) states: “The date of introduction in the southern coast Spanish saltmarshes remains

	uncertain, it was probably introduced between 1970 and 1973 (Fernández-Delgado, 1989). Although Gutiérrez-Estrada *et al*. (1998) suggested some limitations (see below), they did not exclude the early 1970s as the most likely date of introduction. Almaça (1995) had no suggestion regarding the date of introduction of *F. heteroclitus* in the Portuguese side of the Guadiana saltmarshes because fish research at the mouth of the Guadiana only took place after 1975, and thus it could have been present for a long time in this region without being reported. By the 1990s, it was already well established in the southwestern coast of Spain, where it could be found almost continuously from the mouth of the Guadiana until the Barbate marshes (Gutiérrez-Estrada *et al.*, 1998). A decade later, its presence was recorded in the Ria Formosa, southern coast of Portugal (at least since 2002 in seabird pellets; e.g., Catry *et al.*, 2006; Paiva *et al.*, 2006) and in the Ebro Delta in the Mediterranean Sea, north-eastern coast of Spain (Gisbert & López, 2007)" (Figure A8).  Figure A8. Timeline of observations of *Fundulus heteroclitus* in Iberian Peninsula.
A9. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	**Current climate**: This species has a wide latitudinal range in its native distribution (see section A7). It could establish in most EU member states with a marine coast, i.e. Belgium, Bulgaria, Croatia, Cyprus, Denmark, France, Germany, Greece, Italy, Ireland, Malta, the Netherlands, Poland, Romania, Slovenia, the United Kingdom and possibly Estonia, Finland, Latvia, Lithuania, and Sweden.

	Future climate: This species has a wide latitudinal range in its native distribution and climate change should not change much its establishment probability (see section A7). Therefore, under foreseeable climate change it could establish in most EU member states with a marine coast, i.e. Belgium, Bulgaria, Croatia, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Ireland, Latvia, Lithuania, Malta, the Netherlands, Poland, Romania, Slovenia, Sweden and the United Kingdom.
A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?	The existing ecological risks assessments report impacts in Iberian fresh waters but not for the US introductions. This species has barely been introduced outside Europe so there are no impacts reported elsewhere.
A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?	Freshwater / terrestrial biogeographic regions: • Mediterranean Marine regions: • North-east Atlantic Ocean, Mediterranean Sea Marine subregions: Bay of Biscay and the Iberian Coast, Western Mediterranean Sea. See section A7.
A12. In which EU member states has the species shown signs of invasiveness?	Portugal and Spain.
A13. Describe any known socio-economic benefits of the organism.	*Fundulus heteroclitus* is used as ornamental, as bait in sport fisheries, for biological control agents of mosquito larvae (FAO, 2016) and for scientific research. The species is able to tolerate extreme chemical (contamination) and physical conditions (temperature, salinity, oxygen, etc.) (Hardy Jr, 1978; Bulger, 1984) and is easy to reproduce in captivity. For this reason, mummichog is commonly used in scientific research of stress biology, thermal physiology, toxicology, developmental biology, endocrinology, cancer biology genetics or chronobiology and is considered a model species; it is supposed to be the only freshwater fish species used in a space experiment (Bailey *et al.*, 1996; Hawkins *et al.*, 2003; Law, 2001; Walter & Kazianis, 2001; Winn, 2001; Kent *et al.*, 2009). Gutiérrez-Estrada *et al.* (1998) state that "*F. heteroclitus* is consumed in large quantities by very important commercial fish species, such as large *Sparus aurata* and *Dicentrarchus labrax* (Arias, pers. comm.)." of the Atlantic coast of Spain.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: "No information has been found."
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document[2] and the provided key to pathways[3].
- 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	medium	In the Iberian Peninsula (IP), where the mummichog is locally dominant in abundance, the introduction pathways are unclear (Gutiérrez-Estrada *et al.*, 1998; Morim et al. 2019; see below for further details) but

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

pathways respond N/A and move to the Establishment section)			might be multiple and transferable to the risk assessment area.
1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities. For each pathway answer questions 1.3 to 1.10 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.	**A) ESCAPE FROM CONFINEMENT** (Pet / aquarium / terrarium) **B) ESCAPE FROM CONFINEMENT** (Research & ex-situ breeding) **C) TRANSPORT – CONTAMINANT** (Contaminated bait, Contaminant on animals) **D) TRANSPORT - STOWAWAY** (Ship/Boat ballast water)		Killifishes (a common term vaguely used mostly for oviparous cyprinodontiforms) are very popular aquarium fish (Wildekamp, 1993), with several existing hobbyist associations (e.g. http://www.bka.org.uk, https://www.sekweb.org/index_en.php); however, *F. heteroclitus* seems not present in the trade and rarely used by aquarium hobbyists. See below for further details. Similarly, *Fundulus heteroclitus* is a model species used extensive in experimental research, including European laboratories. See 1.3b for examples and justification of the current relevance of this pathway. In the USA, the introductions were mostly as bait bucket releases (U.S. Fish and Wildlife Service, 2017) and in Hawaii for mosquito control (FAO, 2016; Froese & Pauly, 2016). The importation of this particular species for mosquito control or bait seems unlikely, but it could be imported as a contaminant in live bait (see below). Its use as bait exists in the risk assessment area as reported in some Spanish websites (e.g. http://www.surfcastingcadiz.com/seccion_cebos/el_fundulo.html) but corresponds to spread (movement of an organism within the risk assessment area) rather than introduction to the risk assessment area, given the definitions above. This species might be introduced as a contaminant in tanks and containers of live fish importations. It has also been hypothesized that mummichog was

			introduced through ballast water in the southern Iberian Peninsula (see below), so it might also enter as a stowaway (Ship/Boat ballast water).
Pathway name:	**A) ESCAPE FROM CONFINEMENT** (Pet / aquarium / terrarium)		
1.3a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional	high	Killifishes (a common term vaguely used mostly for oviparous cyprinodontiforms) are very popular aquarium fish (Wildekamp, 1993), with several existing hobbyist associations existing in Europe (e.g. http://www.bka.org.uk, https://www.sekweb.org/index_en.php). The mummichog *F. heteroclitus*, which is also called the common killifish, is not a popular species because it is not as beautifully coloured as other species in the group. Although Maceda-Veiga et al. (2013) did not detect this species in some European wholesalers and retailers and its transport and commerce is now forbidden in Spain since it is included in the National black list (Catálogo Nacional de Especies Invasoras), FishBase (Froese & Pauly, 2016) lists F. heteroclitus as in the aquarium trade. According to the European Pet Organization (the Netherlands) and Ornamental Fish International (the Netherlands), this species is "not sold by our sector in the EU. This opinion is supported by Ornamental Fish International who advise that no trade in this species has been reported by any of its members. We also advise that it does not appear to be a species kept by the hobbyist community in the EU." (personal communication). However, by

			October 2019, at least 5 *Fundulus* species (including "*Fundulus* s.p." (sic)) are listed as available from Spanish aquarium hobbyists (https://www.sekweb.org/censo/index.php?letra=f). And Youtube videos (e.g. https://www.youtube.com/watch?v=zp7_N_y77vI&t=31s, last accessed October 2019) demostrates that ¡¡it is sometimes kept in captivity (apparently in Portugal in the video). Misdentification is easy and therefore although this species seems barely present in the aquarium trade, its importation by aquarium hobbyist seems not impossible. Moreover. *F. heteroclitus* is an intertidal spawner and its eggs resist desiccation for several days (Taylor 1999), what would make the importation of dry eggs in packages possible. This pathway is intentional (the organism would be imported for trade or use) (see also Fig 1 in the Guidance document).
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.	unlikely	medium	Maceda-Veiga *et al.* (2013) did not detect this species in some European wholesalers and retailers (see 1.3a). According to the European Pet Organization (the Netherlands) and Ornamental Fish International (the Netherlands), this species is "not sold by our sector in the EU. This opinion is supported by Ornamental Fish International who advise that no trade in this species has been reported by any of its members. We also advise that it does not appear to be a species kept by the hobbyist community in the EU." (personal communication). However, the mummichog "is the most abundant resident fish in most of the salt marshes on the east coast of the United States" (Teo & Able, 2003). Moreover, it is a small-sized, hardy fish that can be transported in small volumes of water. Therefore, the

			movement of large numbers seems unlikely but not impossible.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	The fish could escape from aquarium fish farms or be released as un undesirable pet (e.g. after growing to a certain size). Aquarium fish are sometimes released in the wild by aquarium hobbyists (e.g. this is probably how the guppy established in thermal springs in Spain Hungary and elsewhere) or escape from aquarium facilities. Morim et al. (2019) discuss several possible mechanisms of the first introduction to Europe (southern Iberia) and suggest that aquarium trade is the most likely.
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	moderately likely	medium	The risk of introduction and entry exists.
Pathway name:	**B) ESCAPE FROM CONFINEMENT** (Research & ex-situ breeding)		
1.3b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)? (if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)	intentional	high	*Fundulus heteroclitus* is an experimental model species used extensively in research. Although "countless mummichogs have been hatched in the laboratory, the species has rarely been bred in captivity, that is, propagated from generation to generation." and "it is not widely available like the goldfish, is not easily bred in aquaria like the live bearing guppy" (Atz, 1986). Therefore, the specimens used in the laboratory probably originate largely from wild populations or are imported or bought, so the introduction ("movement of the species into the risk assessment area") is intentional although the entry ("release/escape/arrival in the environment, i.e. occurrence in the wild") would likely be unintentional. In the Ebro delta, this species might have been introduced "from southwestern Spain for research purposes, since this species was used as a biological model in an Aquaculture Research Centre from 2001

			up to middle 2004. Although the wild specimens were found within c. 2 km of the IRTA, containment measures had been undertaken at these research facilities in order to minimize any risk of escape of any developmental stage of *F. heteroclitus* (from egg to adult)" (Gisbert & López, 2007). Other authors are more convinced that the mummichog escaped from this research center (Sierra, 2006; Q. Pou-Rovira, personal communication). Examples of recent research using this species in Europe are Tingaud-Sequeira *et al.* (2009), Lombardo *et al.* (2011, 2012), which seem to have obtained the individuals from southern Spain. Its transport and commerce is now forbidden in Spain since it is included in the National black list (Catálogo Nacional de Especies Invasoras), unless a specific permit is given. Therefore, importation from outside Europe either for research or aquarium purposes should not be difficult at present and possible.
1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.	moderately likely	low	The movement of large numbers is moderately likely.
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	The entry to the Ebro delta was possibly through escapements from an Experimental Research Centre, so it seems moderately likely
1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	moderately likely	medium	The risk of introduction and entry seems to clearly exist.
Pathway name:	**C) TRANSPORT - CONTAMINANT** (Contaminated bait, Contaminant on animals)		
1.3c. Is introduction along this pathway intentional (e.g.	unintentional	high	It could be transported as a contaminant of live bait or

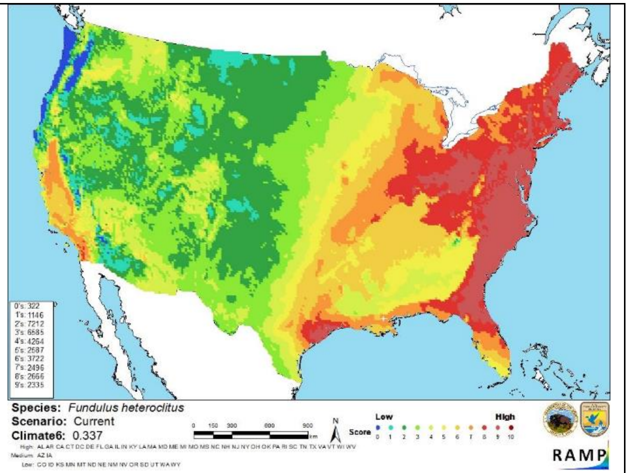
the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?			contaminated animals (other fish species to be farmed or stocked) and these pathways is unintentional. For instance, one reviewer mentioned that it could be unintentionally "spread by juvenile fish, which are caught in one EU member state and then released in the outer waters of another EU member state with the purpose of fish species conservation (for example for the eel *Anguilla anguilla*) or aiming at increasing local fish populations for anglers. Glass eels are for example caught in French and Spanish estuaries and released in the Netherlands, for conservation purposes (e.g., Dekker, W. & L. Beaulaton, 2016. Faire mieux que la nature? The history of eel restocking in Europe. Environment & History 22/2: 255-300). Killifish could mistakenly be transported together with these eels." It is not know if the eggs of F. heteroclitus resist passage through the gut contents of vertebrates, as it was the case of a recent killifish (Silva et al. 2019) but it this was the case and the envolved animals were transported by humans, this could also be part of this passage (contaminant on animals). Note that in North America the eggs of F. heteroclitus develop out of water after high tides (see 1.22).
1.4c. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.	moderately likely	low	*F. heteroclitus* is a small-sized, hardy fish, very abundant in eastern North America. Since live bait (fish and other animals) are transported at the global scale, this species could easily travel as a contaminant.
1.5c. How likely is the organism to survive during passage	very likely	high	The species is considered to be well adapted to

along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.			environmental changes as long as a wide range of salinities (0 to 120.3 ppm) and temperatures (-1.5 to 36.3 ºC) (Griffith, 1974; Umminger, 1972; Garside & Chin-Yuen-Kee, 1972). The organism survives abrupt changes in both parameters (Bulger, 1984; Hardy Jr, 1978). It seems possible but unlikely that the species could reproduce during transport.
1.6c. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	medium	It could get unnoticed or unchecked by border controls.
1.7c. How likely is the organism to enter the risk assessment area undetected?	likely	medium	*F. heteroclitus* is a small fish that could easily enter the risk assessment area undetected.
1.8c. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	high	Mummichog is a hardy species so it could survive and establish any time of the year in suitable climates.
1.9c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	unlikely	low	If the bait is for an open aquaculture facility it could escape and reach a suitable habitat
1.10c. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	unlikely	low	The introduction through this pathway seems moderately likely but the entry unlikely
Pathway name:	**D) TRANSPORT - STOWAWAY** (Ship/Boat ballast water)		
1.3d. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?	unintentional	high	It could be transported through ballast water (see below) and this introduction is unintentional
1.4d. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.	moderately likely	low	In the southern IP, the mummichog was originally introduced in the marshes of the province of Huelva in the early 1970s, with individuals coming from the northern area (Nova Scotia) of its natural distribution range (Bernardi *et al.*, 1995). The way in which this introduction was accomplished is unclear (Gutiérrez-Estrada *et al.*, 1998) but it has been hypothesized that it could have been introduced through ballast water (Sierra, 2006; García-Revillo & Fernández-Delgado 2009, Gonçalves *et al.* 2017), as several invertebrates

			present in the Guadalquivir river (e.g. *Eriocheir sinensis*, *Rithropanopeus harrisii*, *Haliplanella lineata*) (García-Revillo & Fernández-Delgado 2009). However, there is no direct evidence for this and although introduction of fish with ballast water is frequent (Hutchings, 1992; Williams *et al.*, 1988; Wonham *et al.*, 2000), we found no information of clear introductions or detections in ballast water for mummichog. For example, in their extensive global review, Wonham *et al.* (2000), reported 31 fish species detected in ballast water (but not mummichog) and 24 established introductions attributed to ballast water, which included three cyprinodontid fish species, but not the mummichog. *F. heteroclitus* "is the most abundant resident fish in most of the salt marshes on the east coast of the United States" (Teo & Able, 2003) and thus accidental transport with ballast water in large numbers seems moderately likely, although we found limited evidence of it.
1.5d. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	high	The species is considered to be well adapted to environmental changes such as a wide range of salinities (0 to 120.3 ppm) and temperatures (-1.5 to 36.3 ºC) (Griffith, 1974; Umminger, 1972; Garside & Chin-Yuen-Kee, 1972). The organism survives abrupt changes in both parameters as well (Bulger, 1984; Hardy Jr, 1978). "The single attribute of the mummichog that has been most responsible for its remarkable popularity as a laboratory animal is its hardiness in captivity." (Atz, 1986).
1.6d. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	medium	F. heteroclitus is a small-sized, euryhaline fish so it could survive management practices related to exchanges of ballast water with different salinities.
1.7d. How likely is the organism to enter the risk	likely	medium	*F. heteroclitus* is a small-sized fish that can could thus

assessment area undetected?			easily enter the risk assessment area undetected.
1.8d. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	high	Mummichog is a hardy species so it could survive and establish any time of the year in suitable climates
1.9d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	If the discharge of ballast water occurs in a suitable habitat for the species (e.g. estuaries or coastal areas), it seems likely to establish. However, this seems to have occurred in few areas so we scored it as moderately likely
1.10d. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	moderately likely	low	Despite the mummichog being the "ideal" fish species to be introduced with ballast water (small, hardy, abundant in a large native area), this has not occurred many times given the few existing introduced populations.
End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	likely	medium	This species is very abundant in the native areas, very hardy, and could be transported by several pathways. Although not widely introduced worldwide, it is likely to entry into the risk assessment area based on all active pathways. The likelihood is similar in different biogeographical regions except the ones without coastal areas (e.g. Pannonian region).
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	likely	medium	Climate change is not expected to affect much this species (see A7) or its overall likelihood of entry into the risk assessment area.

PROBABILITY OF ESTABLISHMENT			
Important instructions: • For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established. If the species is established in all Member States, continue with Question 1.16.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very likely	high	*F. heteroclitus* is a very tolerant species in terms of temperature and salinity (Griffith, 1974; Umminger, 1972; Garside & Chin-Yuen-Kee, 1972). Its original range includes much of the east coast of USA and Canada, mainly in brackish or saltwater, and it inhabits sheltered coastal areas such as saltmarshes, tidal creeks, estuaries, or bays. In these coastal habitats, it could easily establish in a wide latitudinal range (see Fig. A7 for a map with the potential distribution). Another climate matching map of the species in the USA is available (Fig. 1.13), although it does not seem very reliable since mummichog is mostly a brackishwater species.

			 Figure 1.13. Map of climate matches for *Fundulus heteroclitus* in the contiguous United States based on source locations reported by Fuller (2018) and GBIF. Figure obtained from U.S. Fish and Wildlife Service (2017).
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it and the organism's current distribution?	very likely	medium	*F. heteroclitus* is very tolerant to diverse abiotic conditions (See comments to Q1.13 above and elsewhere) and it has already established in the risk assessment area (Portugal and two separate areas in Spain), although it took decades to establish new populations (apparently because low spread, see below). It seems likely establish in many other countries, although population specific differences might explain that the species has not spread to other EU member states since its establishment in Spain and Portugal.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	widespread	medium	*F. heteroclitus* prefers salt marshes with brackish water but can tolerate freshwater and a range of temperatures so it could establish along much of the European coast and most climates of the risk assessment area. It seems to be limited by the

			existence of benthic muddy saltmarsh environments, which are only found near major estuaries or lagoons areas (Morim et al. 2019) (see A7 above).
1.16. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?	NA	high	There is no known particular species necessary for critical stages in its life cycle.
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very likely	high	*F. heteroclitus* "is the most abundant resident fish in most of the salt marshes on the east coast of the United States" (Teo & Able, 2003) and has established and is abundant in some parts of the Iberian Peninsula (Gutiérrez-Estrada *et al.*, 1998) so competition is unlikely to prevent establishment.
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very likely	high	*F. heteroclitus* "is the most abundant resident fish in most of the salt marshes on the east coast of the United States" (Teo & Able, 2003) and has established and is abundant in some parts of the Iberian Peninsula (Gutiérrez-Estrada *et al.*, 1998) so biotic interactions are unlikely to prevent establishment. There are generic studies on infectivity of *A. invadans* (epizootic ulcerative syndrome) and viral haemorrhagic septicaemia virus (ectoparasites) (Johnson *et al*., 2004; Gagné *et al.*, 2007; Bailly, 2009). No studies have been found of parasites on the Mummichog in the risk assessment area.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very likely	medium	Control experiences of the species by means of passive methods such as fishing net or pots have not served to limit the establishment of the species in the eastern Iberian Peninsula (Pou i Rovira, 2008). If released intentionally or accidentally, it is

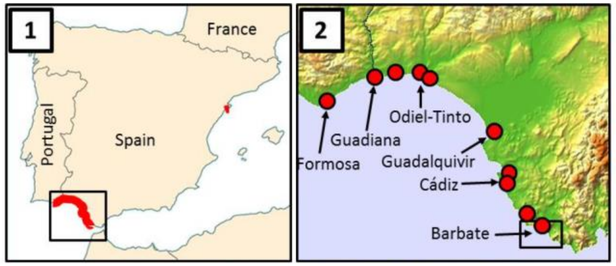
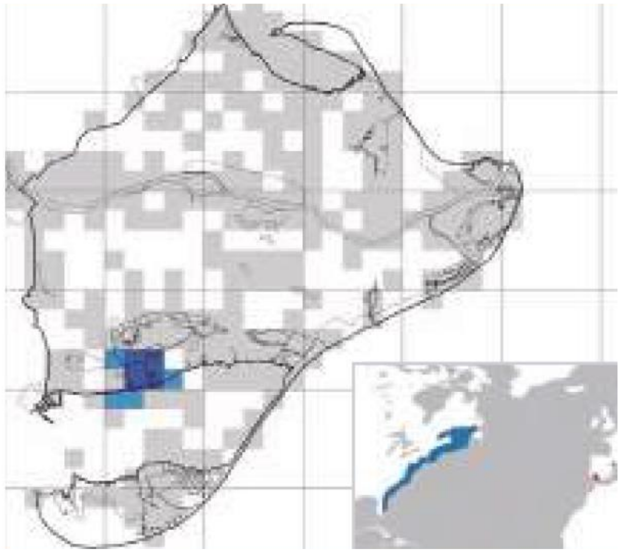
			likely to establish.
1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?	moderately likely	low	In Spain, the transport and commerce of this species is forbidden since it is included in the National black list (Catálogo Nacional de Especies Invasoras). However, current management practices in Spain have not limited the establishment of new fish species in the last 20 years, since there is much illegal or unnoticed fish movement. This is probably the case in other European countries.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	likely	medium	This is a very abundant, small-sized, hardy fish, with ideal properties to resist eradication campaigns in the risk assessment area.
1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?	very likely	high	This is a very abundant, small-sized, hardy fish, with ideal properties to facilitate its establishment in the risk assessment area. *F. heteroclitus* are gregarious and live up to 4 years. It reaches sexual maturity about 35 mm SL and about 1 year. Spawns in April-June in European waters. Eggs are spawned one by one, adhere to vegetation by filaments, and hatch in 12-14 days (Kottelat & Freyhof, 2007). *F. heteroclitus* feed mostly on small crustaceans and polychaetes. Fish longer than 30 mm also ingest considerable living plants (Kneib & Stiven, 1978). Kneib & Parker (1991) conducted experiments about gross food in larval mummichogs and they suggested that natural prey concentration is decisive for fish growth. It feeds at surface, mid-water, and off bottom, mainly at high tide during daylight, but also opportunistically (Abraham, 1985).

			In its native area, *F. heteroclitus* needs an annual reproductive cycle containing lunar and semilunar spawning cycles in January (Hsiao *et al.*, 1994). The species shows a large primary spawning peak in spring followed by a smaller secondary one in mid-summer (Kneib & Stiven, 1978). The eggs are usually located in places covered by high spring tides, usually in sand (Taylor, 1986). Eggs are normally incubated in the air (essential for survival) until the next spring tide. Decreases in salinity from spring rains can decrease the success of fertilization and increase larval mortality (Able & Palmer, 1988). *F. heteroclitus* in aquaria may lay up 40 egg/day depending on size, with some females spawning almost daily throughout the season (Foster, 1967). In field populations, conditions are rarely optimal so that the number of eggs spawned per day is reduced (Kneib & Stiven, 1978). Hatching of most eggs was estimated to occur in May. The main growing season is from April to September. The species grows rapidly with females sexually mature (30-35 mm) in 5-6 months. Mortality in females increases dramatically after the first reproduction at the end of the second growing season (Kneib & Stiven, 1978).
1.23. How likely is the adaptability of the organism to facilitate its establishment?	very likely	high	This is a very adaptable species (to brackish waters), what is likely to facilitate its establishment. In its native area (North America), *F. heteroclitus* are non-migratory, and the movement of individuals is usually localised, limited to relatively small areas, with some individuals

			occasionally dispersing over longer distances. The organism makes small movements between summer and winter habitats with lower salinity areas (Smith & Able, 1994). There are several possible advantages to remaining in the saltmarsh pools during the winter. They are shallow, which allows rapid increases in water temperature. On sunny days in winter, *F. heteroclitus* are active, and temperature increases may be high enough to allow feeding during the day. Small increases in water temperature have been shown to increase *F. heteroclitus* metabolism, especially at water temperatures below 5 °C. In addition, there is little water flow in marsh pools in winter, so fish are not forced to expend energy maintaining their position as they would in the tidal creek (Smith & Able, 1994).
1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?	likely	medium	Studies of genetic diversity of *F. heteroclitus* in Spain were made by Bernardi *et al.* (1995) and Morim et al. (2019). Bernardi *et al.* (1995) have tried to determine from which of the American populations the Spanish individuals are derived. Their results seem to indicate a low genetic diversity for the Spanish population similar to a northern population of North America. Morim et al. (2019), including a sample from the Ebro delta, confirmed the lack of genetic structure and the likely introduction of a few individuals. However, the species has established and is abundant in some parts of the Iberian Peninsula.
1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	moderately likely	low	The species is already established and abundant in the Iberian Peninsula but has almost not established introduced populations in other places worldwide; this seems more related to its transport probability and propagule pressure rather than its

			establishment capacities. It is tolerant to a variety of environmental conditions and very abundant in its native area.
1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur? 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.	unlikely	medium	See comments provided to Q1.25. If introduced, it is likely to establish.
1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	very likely	high	This is a very abundant, small-sized, hardy fish, with ideal properties to facilitate its establishment in most coastal areas of the risk assessment area. It has not a long history of introductions but it is established and abundant in the Iberian Peninsula. If introduced, it is likely to establish in the following Freshwater / terrestrial biogeographical regions under current climate: Freshwater / terrestrial biogeographic regions: Atlantic, Black Sea, Boreal, Continental, Mediterranean, and Steppic. It is likely to establish in the coastal area of the four marine regions (i.e. Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, and Black Sea). See A7 for further info.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	very likely	high	This is a species with a wide latitudinal range in its native area and tolerant of contrasting temperatures and different abiotic factors so climate change should not affect it much. Therefore, climate change should not affect (possibly reinforce) its likelihood of establishment, which is already high much of the coastal areas of the risk assessment area.

PROBABILITY OF SPREAD			
Important notes: • Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area. • Repeated releases at separate locations do not represent spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic "spread" via release or escape should be dealt within the introduction and entry section.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
2.1. How important is the expected spread of this organism within the risk assessment area by **natural** means? (Please list and comment on each of the mechanisms for natural spread.)	minor	high	There has been natural spread within the two introductions in the Iberian Peninsula. In the southern Iberian Peninsula, it has spread slowly since its introduction supposedly in the 1970s (Fig. 2.1a), presumably by natural dispersal, since this species is a euryhaline species that has been shown to be able to use marine environment as dispersal routes (Blanco-Garrido & Clavero, 2016). In a 1-year mark-recapture study in Canada, 97% of recaptured fish were within 200 m of the point of initial release, whereas the rest moved distances ranging from 600 to 3600 m (Skinner *et al.*, 2005). Similarly, since its introduction in a single site of the Ebro delta in 2005 it has spread slowly in the delta (Fig. 2.1b).

			 Figure 2.1a. Distribution and recent expansion of the mummichog (*Fundulus heteroclitus*) in the Iberian Peninsula. 1) Current distribution of the mummichog in the Iberian Peninsula marked with a red line. 2) Main population nuclei of the mummichog in southern Iberian Peninsula (reproduced from Blanco-Garrido & Clavero, 2016).  Figure 2.1b. Distribution of the mummichog (*Fundulus heteroclitus*) in the Ebro delta ca. 2012 (the

			native distribution in North America is also shown) (reproduced from López *et al.*, 2012). Mummichogs make daily tidal migrations between the intertidal marsh surface and adjacent channel and pond habitats (Butner & Brattstrom, 1960; Weisberg & Lotrich, 1982) and, as a result, are hypothesized to play an important role in the export of marsh production to the open estuary (Kneib, 1997). Despite these movements, mummichogs are thought to have a highly restricted summer home range of only 36 m (Lotrich, 1975). However, it was found that in a restored salt marsh, YOY and adults primarily used the shallow subtidal and intertidal areas of the created creek, the intertidal drainage ditches, and the marsh surface of the restored marsh but not the larger, first-order natural creek. At low tide, large numbers were found in the subtidal areas of the created creek; these then moved onto the marsh surface on the flooding tide. Elevation, and thus hydroperiod, appears to influence the microscale use of the marsh surface. So in other studies the home range of adults and large YOY has been estimated to be 15 ha at high tide, much larger than previously quantified (Teo & Able, 2003). There was strong site fidelity to the created creek at low tide. The habitat uses and movement patterns of the mummichog appeared similar to that reported for natural marshes (Teo & Able, 2003). The eggs of an annual Brazilian killifish, which are very adapted to dessication and diapause, have been recently shown (Silva et al. 2019) to resist the passage of bird guts and might thus disperse with birds and other animals. However, it is unclear if this applies to other cyprinodontiforms.

2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	minor	medium	The two intentional pathways of introduction and entry analysed above (ESCAPE FROM CONFINEMENT: Pet / aquarium / terrarium; ESCAPE FROM CONFINEMENT: Research & ex-situ breeding) might also explain "spread" within the risk assessment area but should not be considered as such according to the instructions above. For instance, one of these two pathways would explain the introduction to the Ebro Delta (transport by car/road from southern Spain) but it is "intentional anthropogenic "spread" via release or escape [and] should be dealt within the introduction and entry section" (see above). The slow recent spread in the southern Iberian Peninsula was suggested to be most probably by natural spread through the sea. Although a human-assisted expansion is less likely it is also possible, e.g. through bait releases (Blanco-Garrido & Clavero, 2016, Q. Pou_Rovira, personal communication).
2.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 2.3 to 2.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.	**TRANSPORT - CONTAMINANT** (Contaminated bait, Contaminant on animals) **TRANSPORT - STOWAWAY** (Ship/Boat ballast water)		See below for justification and some information on the specific origins and end points of the pathways.
Pathway name:	**A) TRANSPORT - CONTAMINANT** (Contaminated bait, Contaminant on animals)		
2.3a. Is spread along this pathway intentional (e.g. the	both	high	It could be transported as a contaminant of other taxa

organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?			used for aquaculture or angling. Anglers are frequent nearby sites in Spain where mummichog has been introduced and is abundant and could easily be used as bait and released (Q. Pou-Rovira, pers. comm.). *F. heteroclitus* can be extremely abundant in areas of SW Spain with important semi-captive production of *Sparus aurata*, *Dicentrarchus labrax* and other market valued fish species. These same species are produced in several other areas within the EU. It is thus plausible that any fish movement among aquaculture facilities may involve the movement of mummichog as a contaminant (M. Clavero, pers. comm.).
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?	moderately likely	medium	A few fish could originate a viable population that would spread along this pathway.
2.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	high	Very hardy fish (see Q 1.5d and elsewhere). It is likely to survive. Reproduction during passage along the pathway seems unlikely.
2.6a. How likely is the organism to survive existing management practices during spread?	moderately likely	medium	Very hardy fish; moderately likely to survive existing management practices during spread
2.7a. How likely is the organism to spread in the risk assessment area undetected?	moderately likely	medium	*F. heteroclitus* is a small-sized fish that can could thus easily spread the risk assessment area undetected.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	moderately likely	medium	It could spread to any saltmarsh or estuary nearby, which are widespread but a quite specific habitat. Fig. 2.8a shows the main salt marshes in the European Union, where it could spread.

			 Figure 2.8a. Distribution of saltmarsh in Europe (reproduced from Boorman, 2003).
2.9a. Estimate the overall potential for spread within the Union based on this pathway?	slowly	medium	Given the case of the Iberian Peninsula, it is quite likely that the species will spread further into Europe but quite slowly and not necessarily with this pathway
Pathway name:	**B) TRANSPORT - STOWAWAY** (Ship/Boat ballast water)		
2.3b. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	unintentional	high	It could be transported through ballast water within the risk assessment area and this pathway is unintentional
2.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?	moderately likely	medium	Accidental transport with ballast water within the risk assessment area seems moderately likely. We found no direct evidence of transport or introduction of F. heteroclitus through ballast water (see Q 1.4d). However, the mummichog is abundant in southern Spain, where boats enter the Guadalquivir to mostly

			discharge containers in Seville. Therefore these boats export ballast water and stowaway species rather than import them (García-Revillo & Fernández-Delgado 2009) and could favour spread to other European ports and coastal areas.
2.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	high	Very hardy fish. See Q 1.5b and elsewhere. Reproduction during passage along the pathway seems unlikely.
2.6b. How likely is the organism to survive existing management practices during spread?	moderately likely	medium	*F. heteroclitus* is a small-sized, euryhaline fish so it could survive management practices related to exchanges of ballast water with different salinities or other management practices.
2.7b. How likely is the organism to spread in the risk assessment area undetected?	moderately likely	medium	It should not take very long to detect if there are fish surveys in the region but it could take months to years if not. It would probably spread slowly.
2.8b. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	likely	high	Its arrival location would probably be quite suitable and it could spread to any saltmarsh or estuary nearby.
2.9b. Estimate the overall potential for spread within the Union based on this pathway?	slowly	medium	Given the information available (worldwide history and the case of the Iberian Peninsula), it seems quite likely that the species will spread further into Europe but quite slowly and not frequently.
End of pathway assessment, repeat as necessary.			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	difficult	high	If introduced and established in the risk assessment area, it would likely be difficult and probably impossible to contain *F. heteroclitus* to avoid further spread because this species generally occupies large, open areas (mostly estuaries, coastal lagoons, or similar). When detected as established it would have probably occupied already a considerable area, since

			it is a small fish with rapid maturation (one year after hatching in southern Iberia), relative long reproductive season (although mostly in March and April in southern Iberia), and high densities (Fernández-Delgado 1989).
2.11. Estimate the overall potential for spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	slowly	high	Given the wide latitudinal range of this species in the native area (see Q A.4), it might spread to many of them, but quite slowly and infrequently (as discussed above). As indicated elsewhere, it could spread to most biogeographical regions of the European Union, namely the Atlantic, Black Sea, Boreal, Continental, Mediterranean, and Steppic Freshwater / terrestrial biogeographic regions and the four marine regions (i.e. Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, and Black Sea). See A7 for further info.
2.12. Estimate the overall potential for spread in relevant biogeographical regions in foreseeable climate change conditions	slowly	high	This is a species with a wide latitudinal range in its native area and tolerant of contrasting temperatures and different abiotic factors so climate change should not affect it much. Therefore, climate change should not affect much (possibly reinforce) its potential for spread in the many biogeographical regions where it could spread (see Q2.11).

MAGNITUDE OF IMPACT

Important instructions:

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

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?	minor	high	*Fundulus heteroclitus* was introduced to Hawaii and The Philippines but apparently did not establish there, so there is virtually no other introduced populations than those in Spain and Portugal and a few drainages in the USA, where there are no known reported impacts
2.14. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?	major	medium	The Iberian Peninsula has three endemic, threatened cyprinodontiforms: *Aphanius iberus* (Valenciennes, 1846), *Valencia hispanica* (Valenciennes, 1846), and the recently described *Aphanius baeticus* Doadrio, Carmona y Fernández-Delgado, 2006. *Aphanius baeticus* in southern Spain and *Aphanius iberus* in the Mediterranean Spain occupy a very similar habitat than *F. heteroclitus*. *F. heteroclitus* poses a potential threat by competition and/or predation of the endemic species, and may act synergistically with habitat destruction resulting in a more profound negative impact (Bernardi *et al.*, 1995;

			Doadrio *et al.*, 2002; Elvira, 1996; Elvira & Almodóvar, 2001; Fernández-Delgado, 1989; García-Berthou *et al.*, 2007; García-Llorente *et al.*, 2008; Leunda, 2010; Oliva-Paterna *et al.*, 2006; Planelles & Reyna, 1996; Morim 2017). Mummichog is often numerically dominant in western Andalusian coastal marshes, and it is suspected that it may have negatively affected native endemic species as the endangered Andalusian toothcarp, *Aphanius baeticus* (Gutiérrez-Estrada *et al.*, 1998). According to Gutiérrez-Estrada *et al.* (1998): "If mummichog were outcompeting other species, the mechanisms of this potential exclusion have not been directly evaluated and remain unknown. However, direct predation does not seem to be a factor because *F. heteroclitus* consumes only invertebrates and plants in the study area (Hernando, 1975; Arias & Drake, 1986). In addition, the competition for food does not seem to be a decisive factor due to the enormous productivity of the areas where it is found. Therefore, perhaps, the competition for space could be the best explanation for this apparent segregation observed for mummichog and other fish species in the study area". "It is difficult to evaluate the precise ecological consequences of the mummichog introduction in southern Iberia, especially due to the fact that the original environmental conditions existing in the area where it was introduced are unknown. However, it is probable that some effects may have been negative. Some local fish species may have been displaced". It seems to be affecting *Aphanius iberus* in the Ebro delta and could spread to freshwaters where *V. hispanica* inhabits (López *et al.*, 2012).

			F. heteroclitus is often numerically dominant in both the native area and its introduced area in southern Iberia.
2.15. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?	major	medium	The potential invaded area of the species is limited to coastal saline areas. The impact in the introduced areas has not been realised since it has spread recently to new areas. It is likely to decrease the conservation status of some these threatened species by decreasing their abundance and range and possibly their genetic diversity. If mummichog arrives to new areas of the risk assessment area, it could affect other threatened species, such as *Aphanius fasciatus* in Mediterranean coastal areas, *Valencia* spp. in Greece and others. The mummichog, *Fundulus heteroclitus*, "is the most abundant resident fish in most of the salt marshes on the east coast of the United States, and, as a result, is a key ecological component" (Teo & Able, 2003). Since it is very abundant in some Iberian populations, it is likely to also play a key ecological role in the food web and ecosystem functioning and change current structure.
2.16. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?	major	high	*F. heteroclitus* mostly inhabits protected areas (e.g. the Doñana National Park or the Ebro Natural Park in Spain, where *F. heteroclitus* is now abundant). It seems to be clearly affecting two threatened species: – *Aphanius baeticus* (EN), – *Aphanius iberus* (EN), The zones inhabited by *F. heteroclitus* are mostly transitional areas according to the Water Framework Directive (WFD); the effects of mummichog for the

			WFD assessment are largely unknown.
2.17. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?	major	medium	The introduction and spread of *Fundulus heteroclitus* in the saltmarshes of the risk assessment area might affect a multitude of native, threatened species and saltmarsh habitat types. In addition, all the marshes of the European Union usually have a degree of protection due to their high ecological uniqueness. The species potentially impacted are included in the IUCN red list or are endemic species (Kottelat & Freyhof, 2007; Freyhof & Brooks, 2011), namely: – *Aphanius almiriensis* (CR), – *Aphanius baeticus* (EN), – *Aphanius fasciatus* (LC), – *Aphanius iberus* (EN), – *Valencia hispanica* (CR), – *Valencia letourneuxi* (CR) – *Valencia robertae* (not yet evaluated) (IUCN categories: CR = Critically Endangered, EN = Endangered, LC = Last Concern and VU = Vulnerable) Except for the species recently described, four of the species abovementioned (*A. iberus*, *A. fasciatus*, *V. hispanica*, and *V. letourneuxi*) are included in Annex II of the Habitats directive (Council Directive 92/43/EEC of 21 May 1992). If *F. heteroclitus* penetrates to low salinity stenohaline environments, it could also affect *Gasterosteus aculeatus* (LC) or *Cobitis paludica* (VU), among many others. Saltmarsh habitat types are protected under Directive

			92/43/EEC on the conservation of natural habitats and wild flora and fauna and specific national or regional legislation.
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	minor	high	*Fundulus heteroclitus* was introduced to Hawaii and The Philippines but apparently did not establish there, so there is virtually no other introduced populations than those in Spain and Portugal and a few drainages in the USA, where there are no known reported impacts
2.19. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?	minor	medium	The impact of mummichog on ecosystem services is caused by possible changes to the food web due resource competition, predations, or spread of disease. This can possibly lead to diminishing of the provisioning of native species for fisheries and quality of nursery habitats. It can also cause changes in ecosystems structure and species composition that make it attractive for recreation, wild life watching etc. Provisioning: In southern Spain, there have been probably negative impacts in traditional prawn fishery yields, which are known to be heavily consumed by mummichog (Arias & Drake, 1986; U.S. Fish and Wildlife Service, 2017). We found no published information on this question but some impacts on regulation and maintenance (e.g. given species abundance and important ecological role) and cultural services are likely.
2.20. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?	minor	low	If it spreads to other European areas, the impacts should be similar than in the Iberian Peninsula but affecting many other species, ecosystems and human populations.

Economic impacts			
2.21. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management	minor	high	*Fundulus heteroclitus* was introduced to Hawaii and The Philippines but apparently did not establish there, so there is virtually no other introduced populations than those in Spain and Portugal and a few drainages in the USA, where there are no known reported impacts
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	minor	low	The economic costs of the mummichog in the Iberian Peninsula has not yet been evaluated but see Q 2.19
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	moderate	low	They have not been well evaluated but do not seem very large. It could affect coastal areas where there are fisheries or aquaculture by changing ecosystem structure and functioning.
2.24. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?	moderate	low	The economic costs associated with the management of the mummichog in the Iberian Peninsula have not yet been evaluated. However, money is spent in monitoring and control the invasive species and to implement further conservation plans for native and endemic, threatened species (maintaining captive stocks, restocking, etc.).
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	moderate	low	While no cost estimates for mummichog are available, information on other species can be used as a proxy. Britton *et al.* (2008) list the cost of eradication by different means and site of *Pseudorasbora parva* and estimated cost of 1.9-7.9 £/m^2 in UK ponds. Given the large, open areas occupied by *F. heteroclitus* in Spain, eradication is probably not feasible in most sites but would cost hundreds of thousands of euros.
Social and human health impacts			
2.26. How important is social, human health or other impact (not directly included in any earlier categories)	minimal	medium	Harmless to humans according to FishBase (Froese & Pauly, 2016).

caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).			However, possible wider societal impacts could arise if the invasion has negative impacts on fisheries and other ecosystem services (see 2.19) and starts to threaten local livelihoods.
2.27. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.	minimal	medium	No information has been found on this issue.
Other impacts			
2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	moderate	low	From Johnson *et al.* (2004): “We explored the infectivity of *A. invadans* (WIC strain) when inoculated into four commonly occurring species: Atlantic menhaden, striped killifish, *Fundulus majalis* (Walbaum), mummichog *F. heteroclitus* (L.), and *hogchoker*, *Trinectes maculatus* (Bloch & Schneider). […] Mummichogs experienced a lower prevalence of lesions compared with the other species. […]” Infection with *A. invadans* (epizootic ulcerative syndrome) is an OIE-reportable disease. From Gagné *et al.* (2007): “Viral haemorrhagic septicaemia virus (VHSV) was isolated from mortalities occurring in populations of mummichog, *Fundulus heteroclitus*, stickleback, *Gasterosteus aculeatus*, brown trout, *Salmo trutta*, and striped bass, *Morone saxatilis*, in New Brunswick and Nova Scotia, Canada.” Viral haemorrhagic septicaemia virus is an OIE-reportable disease. From Bailly (2009):

			"*Caligus rufimaculatus* Wilson C.B., 1905 [via synonym] (parasitic: ectoparasitic) *Ergasilus funduli* Krøyer, 1863 [via synonym] (parasitic: ectoparasitic) *Ergasilus manicatus* Wilson C.B., 1911 [via synonym] (parasitic: ectoparasitic) *Homalometron pallidum* Stafford, 1904 [via synonym] (parasitic: endoparasitic) *Lernaea cyprinacea* Linnaeus, 1758 [via synonym] (parasitic: ectoparasitic) *Lernaeenicus radiatus* Le Sueur, 1824 [via synonym] (parasitic: ectoparasitic) 6 *Swingleus ancistrus* Billeter, Klink & Maugel, 2000 [via synonym] (parasitic: ectoparasitic)"
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	NA		
2.30. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?	moderate	low	Mummichog is abundant in some parts of southern Spain so predators or other enemies do not control their populations.

RISK SUMMARIES

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

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

			two separate areas of the Iberian Peninsula and it is very likely to establish in most coastal areas of the European Union, if introduced. It is rather a sedentary species that has been shown to spread in the Iberian Peninsula although infrequently and slowly. It seems to already impact endemic, endangered Iberian cyprinodontiforms, with less impacts in ecosystem services and reduced economic costs. If introduced to other Mediterranean areas, it is likely to impact other endemic fauna.

Distribution Summary:

Please answer as follows:
Yes if recorded, established or invasive
– if not recorded, established or invasive
? Unknown; data deficient

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

Member States

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

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

Biogeographical regions of the risk assessment area

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

Marine regions and subregions of the risk assessment area

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

Western Mediterranean Sea	Yes	Yes	Yes	Yes

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ANNEX I Scoring of Likelihoods of Events

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

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

ANNEX II Scoring of Magnitude of Impacts

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

Score	**Biodiversity and ecosystem impact**	**Ecosystem Services impact**	**Economic impact (Monetary loss and response costs per year)**	**Social and human health impact**
	Question 2.18-22	*Question 2.23-25*	*Question 2.26-30*	*Question 2.31-32*
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected[4]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

[4] Not to be confused with “no impact “.

	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[5]	**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.*

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

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

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

ANNEX V EU Biogeographic Regions and MSFD Subregions

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

and

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