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

Contract No 07.0202/2016/740982/ETU/ENV.D2

Final Report

Annex 3: Risk Assessment for *Gambusia affinis* (Baird and Girard, 1853)

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2016/740982/ETU/ENV.D2

Based on the Risk Assessment Scheme developed by the GB Non-Native Species Secretariat (GB Non-Native Risk Assessment - GBNNRA)

Name of organism: *Gambusia affinis* (western mosquitofish).

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Risk Assessment Area: Territory of the European Union (excluding the outermost regions) and the United Kingdom

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

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RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Entry	**moderately likely**	**high**	The distribution of responses was effectively bi-modal, with unlikely and likely being the most frequent responses, so the overall response for Entry is 'moderately likely'. Confidence was consistently high.
Summarise Establishment	**very likely**	**high**	*G. affinis* has broad distributions both in their native and introduced ranges outside the risk assessment area, with demonstrated ability to adapt to areas with colder climatic conditions, including some sources mentioning persistence and establishment in water bodies that are subjected to winter ice cover. As such, establishment risk is 'very likely', and confidence is high, based on the breadth of literature available (our search was thorough but not exhaustive, it resulted however to substantial evidence regarding the species ability for adaptation to low temperatures).
Summarise Spread	**Slow**	**Low**	Overall, the spread of *G. affinis* is likely to be slow, and in view of the very limited available evidence for Europe, there is low confidence in this ranking of dispersal speed.
Summarise Impact	**Major**	**low**	Overall, the current and future impacts of *G. affinis* are moderate (economic) to major (biodiversity and ecosystem function), so 'major' seems to be the appropriate classification of impact. Much of the evidence for non-biodiversity impacts is speculative and/or conflicting, so the overall confidence in the responses is low.
Conclusion of the risk assessment	**High**	**low**	The close congener *G. holbrooki* is known to exert many negative impacts and it is highly likely that *G. affinis* will have the same effects.

EU CHAPEAU (EUc)		
QUESTION	**RESPONSE**	**COMMENTS**
EUc-1. In which EU biogeographical region(s) or marine sub-region(s) has the species been recorded and where is it established?	Mediterranean	The only confirmed population of *G. affinis* in the risk assessment area is in a pond near Naples (Italy) (Carella et al. 2013), which falls within the 'Mediterranean' biogeographic region (EEA 2012). See Figure A (see next page).
EUc-2. In which EU biogeographical region(s) or marine sub-region(s) could the species establish in the future under current climate and under foreseeable climate change?	Atlantic, Continental and Mediterranean	Based on the climate zone span in its native range and the adaptive tolerance of the species to colder temperatures (see details below under question EUc-5), the potential biogeographical regions are: Atlantic, Continental and Mediterranean (EEA 2012).
EUc-3. In which EU member states has the species been recorded? List them with an indication of the timeline of observations.	*G. affinis*: Italy only (Vidal et al. 2010; Sanz et al. 2013; Carella et al. 2013). Internet sales in England of *Gambusia* (i.e. *G. affinis*) have been found (page date = 2011), but any papers mentioning wild populations probably misquote historical records, i.e. Wheeler et al. (2004), which reports the presence of another Poeciliidae (guppy *Poecilia reticulata*).	Vidal et al. (2010) stated that historical records and other data suggest that *G. affinis* "was introduced to Italy in 1927 and it might be present". This presence was confirmed by Carella et al. (2013), who provided genetic proof of *G. affinis* in a pond near Cancello Arnone (Campania, Caserta, Italy).

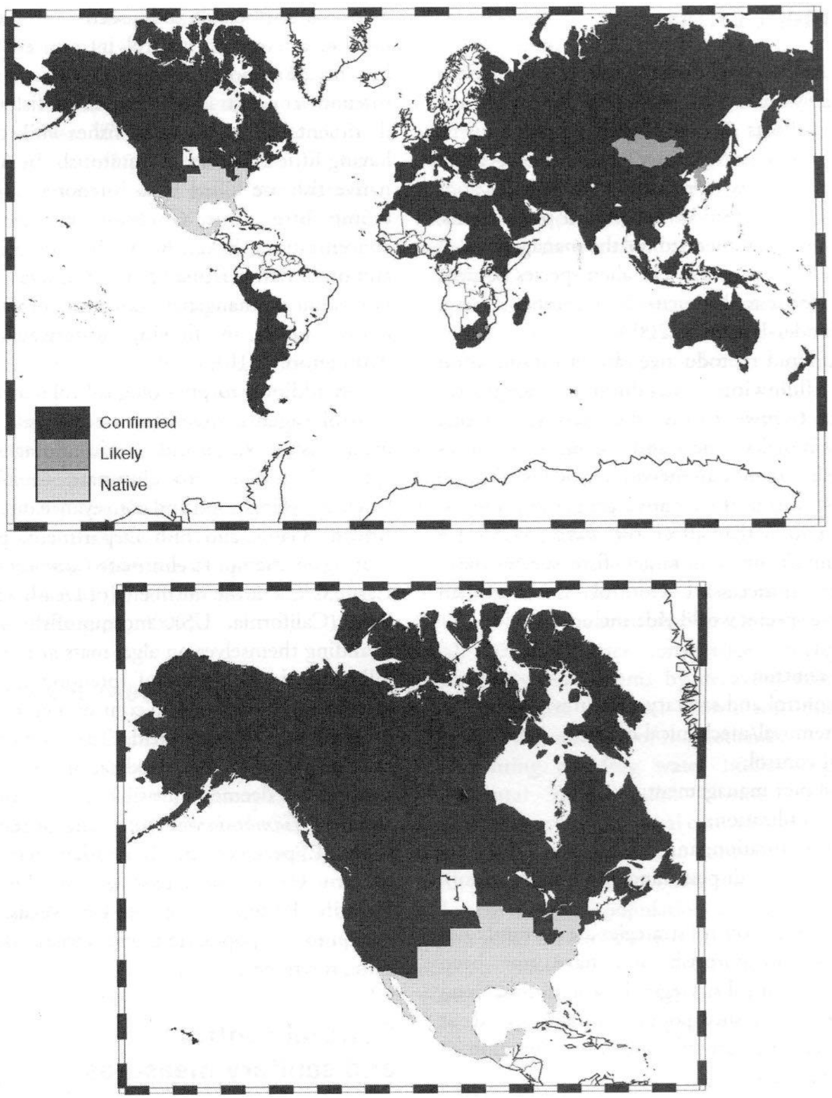


Figure A: Worldwide and North American distributions of *Gambusia* species (Figure 22.3 in Walton et al. 2012).

EUc-4. In which EU member states has this species established populations? List them with an indication of the timeline of establishment and spread.	*G. affinis*: Italy only (Vidal et al. 2010; Sanz et al. 2013; Carella et al. 2013). Timeline of past introductions given in Figure B (here below).	According to Vidal et al. (2010): “Apparently, both species were introduced to Europe in the 1920s (Krumholz 1948) and although mostly *G. holbrooki* is cited, it is unclear whether both species are present. *G. affinis* arrived in Italy directly from the USA around December 1927 (Sella 1926; Anonymous 1927) but despite being cited, it is unclear whether it is still present in Europe (except for the one population in Italy), given the above- mentioned taxonomic problems.” Erroneous determinations are possible though, as *G. affinis* is reported for the salt marshes of the River Loire (France) by Mathieson et al. (2000), whereas Beaudouin et al. (2008) report the cold-adapted population in Brittany, which the Loire passes through, are *G. holbrooki*. Because of the lack of taxonomic clarity prior to Vidal et al. (2010), all prior identifications of *G. affinis* for France are erroneous and must be considered to have been *G. holbrooki* (G. Deny, pers. comm.)

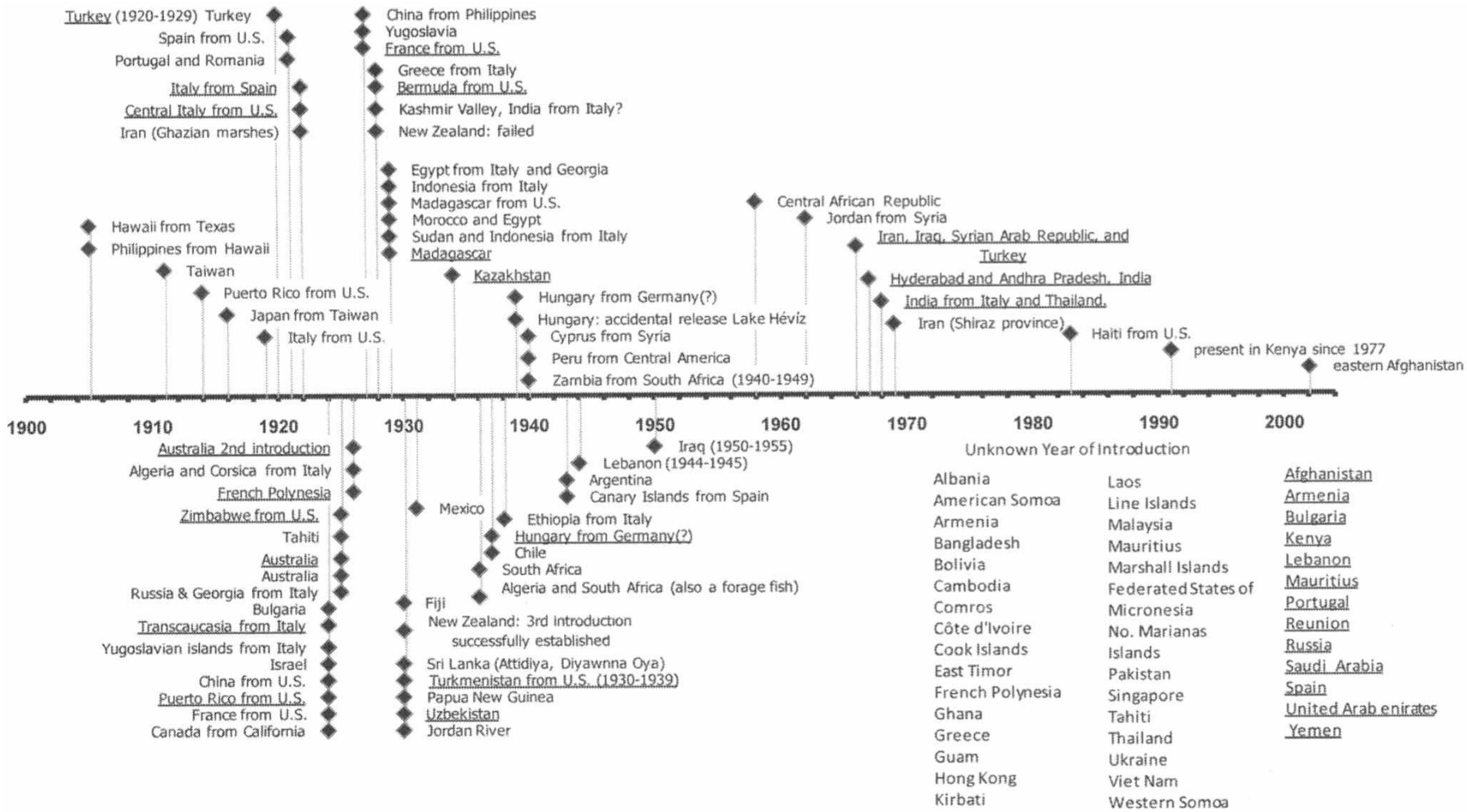


Figure B: Timeline of *Gambusia* introductions worldwide (Figure 22.2 in Walton et al. 2012).

EUc-5. In which EU member states could the species establish in the future under current climate and under foreseeable climate change?	*G. affinis* species have been confirmed to be established already in Italy (see response to Q EUc-3 here above) and the species could probably establish in the following EU countries: Austria (lowland parts), Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, France, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain and UK.	The adaptive tolerance to colder temperatures of *G. affinis* (Otto (1973) indicates potential establishment across a broad area of southern to northerly parts of the EU. Indeed, the *Gambusia affinis* introduced to ponds in Rovigno and Valle d'Istria, Italy, from Carbondale (Illinois, USA) in December 1927 were apparently used because they were thought to be more resistant to cold temperatures than G. *holbrooki* (Vidal et al. 2010). Some documents have ignored existing literature on cold tolerance, stating that the northerly limits of *G. affinis* native ranges are due to their intolerance of water temperatures <4°C (Johnson 2008). However, the review by Haas et al. (2003) points out the earlier studies (Krumholz 1944; Towns 1977) in which *Gambusia affinis* were observed to survive and establish even in ponds that experienced winter ice cover. Most of the western EU falls within the 'Cfb' Köppen-Geiger climate zone (Peel et al. 2007), extending from northern Spain to southern Denmark and Sweden, encompassing all of the British Isles towards the east to the longitudinal mid-point of Germany, with pockets of Cfb zone in northern Italy, in Slovenia, Croatia, Bosnia Herzogovina, Serbia and Bulgaria, Austria, the Czech Republic and even a thin strip of southern Norway. So, given that the native and introduced ranges of *G. affinis* in the USA (Nico et al. 2017) encompasses even colder and warmer/dryer climate zones (Peel et al. 2007) than Cfb, one can assume that *G. affinis* could establish in most of western and southern EU MSs.

EUc-6. In which EU member states has this species shown signs of invasiveness?	*G. affinis* is very localised, limited to at least one pond in Italy, where it is established. Whether or not *G. affinis* is invasive in that part of Italy remains unknown, as the abstract of the study by Carella et al. (2013) does not indicate the number of *G. affinis* captured nor their relative abundance within the pond's fish assemblage.	*G. affinis* are considered to be invasive. There are multitudes of papers from Europe that provide circumstantial evidence for *Gambusia* sp. (e.g. dietary or habitat overlap), but as with most non-native species, there are relative few studies that demonstrate direct impacts. In the case of *G. affinis*' close congener, *G. holbrooki*, there is relatively more evidence for direct adverse impacts, namely on endangered carptooth species (e.g. Rincón et al. 2002; Caiola & de Sostoa 2005; Alcaraz et al. 2008) and the endangered Corfu killifish (Kalogianni et al. 2012).
EUc-7. In which EU member states could this species become invasive in the future under current climate and under foreseeable climate change?	Austria (lowland parts), Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, France, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain and UK (England).	This list comprises Italy, the only country with proven *G. affinis* presence, and a best estimate (based on climate maps (Peel et al. 2007) where this species is likely to become, invasive.

Distribution Summary:

EU Member States and the United Kingdom

	Recorded	Established (currently)	Established (future)	Invasive (currently)
Austria	-	-	Yes	-
Belgium	-	-	Yes	-
Bulgaria	-	-	Yes	-
Croatia	-	-	Yes	-
Cyprus	-	-	Yes	-
Czech Republic	-	-	Yes	-
Denmark	-	-	Yes	-
Estonia	-	-	-	-
Finland	-	-	-	-
France	-	-	Yes	-
Germany	-	-	Yes	-

Greece	-	-	Yes	-
Hungary	-	-	Yes	-
Ireland	-	-	Yes	-
Italy	Yes	Yes	Yes	Yes?
Latvia	-	-	-	-
Lithuania	-	-	-	-
Luxembourg	-	-	Yes	-
Malta	-	-	-	-
Netherlands	-	-	Yes	-
Poland	-	-	Yes	-
Portugal	-	-	Yes	-
Romania	-	-	Yes	-
Slovakia	-	-	Yes	-
Slovenia	-	-	Yes	-
Spain	-	-	Yes	-
Sweden	-	-	-	-
United Kingdom	-	-	Yes	-

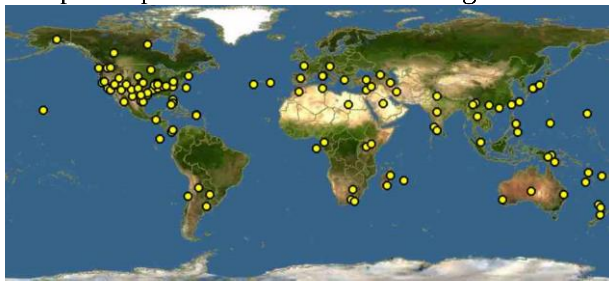
-

EU biogeographical regions

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

SECTION A – Organism Information and Screening		
Organism Information	**RESPONSE** **[chose one entry, delete all others]**	**COMMENT**
1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Western mosquitofish *Gambusia affinis* (Baird and Girard, 1853), which has been known to be distinct from its close congener, the eastern mosquitofish, *G. holbrooki*. Girard, 1859, since the the late 1980s (Wooten et al. 1988).	*G. affinis and G. holbrooki* are two closely-related poeciliid species native to parts of the USA and Mexico, though introduced into >50 countries worldwide (García-Berthou et al. 2005) for mosquito/malaria control. During their worldwide introductions in the early 20th century, mosquitofishes were believed to comprise three species: *G. affinis*, *G. holbrooki* and *G. patruelis*, the latter now considered a synonym of *G. affinis*. Later, *G. affinis* and *G. holbrooki* were considered to be two sub-species of *G. affinis*, then Wooten et al. (1988) designated them as two separate species, which means that many records referring to G. affinis may or are in fact referring to *G. holbrooki* (Haynes and Cashner 1995). Owing to the taxonomic confusion surrounding these two species, their worldwide distribution remains ambiguous (Pyke 2008).
2. Provide information on the existence of other species that look very similar	Although the Genus *Gambusia* is large, comprised of over 40 species, *G. affinis*, and its close congener *G. holbrooki*, are the best known and have been the most widely introduced (for mosquito control). These two species very similar to each other, which explains why they were combined as sub-species of the same taxon, but then later designated as separate species (Wooten et al. 1988). This species can also be easily mis-identified as a guppy species (*Poecilia reticulata*) by anglers (N. Poulet, personal communication)	The two species can be distinguished on the basis of differences in external morphology, chromosome morphology and genetic makeup. They differ in the number of dorsal fin rays, with *G. affinis* having seven and *G. holbrooki* having eight (Pyke 2005).

	and more generally by the general public, which is a concern for endangered toothcarp species such as the Mediterranean banded killifish *Aphanius fasciatus* (Zogaris 2014, 2017).	
3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the EU)	Risk assessments exist for outside the EU and these are at least partially relevant to the EU: Schleier et al. (2008) provides a risk assessment for river basins in Montana, USA; and an assessment of infestation risk for Tasmania (Davies 2012). Schleier et al. (2008) makes specific reference to cold-tolerant strains of *G. affinis*. Numerous risk screenings of *G. affinis* have been undertaken around the world, including for a few EU countries, using the Fish Invasiveness Screening Kit (FISK; Copp et al. 2009), and for and neighbouring countries that at least in part fall within Europe (i.e. Turkey). The risk rankings (MH = moderately high; H = high, VH = very high) were reported as: England & Wales (Copp et al. 2009) **H** Iberia (Almeida et al. 2013) **H** Turkey (Tarkan et al. 2014) **VH** A subsequent risk screening for Turkey, using the Aquatic Species Invasiveness Screening Kit (AS-ISK; Copp et al. 2016), but with a ranking scale of Low (L), Medium (M) and High (H), reported the following ouctomes for current and future climate conditions, respectively: • Turkey (Tarkan et al. 2014) **H, H** • Iran, Anzili Wetland Complex (Moghaddas et al. pers. comm.): **H, H**	Schleier et al. (2008) and Davies (2012) relate to areas outside the risk assessment area, but with temperate climates (i.e. annual temperature minima and maxima) similar to large parts of the EU. The risk screenings for various locations in the EU ranked both species as posing an overall high risk of being invasive, ranging from moderately high (**MH**) risk to high (**H**) risk.

4. Where is the organism native?	North America (USA and Mexico)	Mississippi River basin from central Indiana and Illinois south to the Gulf of Mexico, from the western edges of Georgia, Florida, North and South Carolina and Virginia, westward to Texas and New Mexico. • *G. affinis* in their native range inhabit lowland ponds, lakes and streams, preferring lentic waters, shallows and margins or pools, with dark-coloured silt substrata and dense sub-surface vegetation for lateral rather than vertical cover from potential predators (summarized from Lloyd et al. 1986).
5. What is the global non-native distribution of the organism (excluding the Union, but including neighbouring European (non-Union) countries)?	Too many countries worldwide to list, encompassing virtually all continents except the Antarctic. See www.fishbase.org	*G. affinis* now occurs on every continent except Antarctica as the result of human-assisted movement (Arthur & Subasinghe 2002). See also the species profile at: www.fishbase.org  Figure 1_Q5: Map of *Gambusia* spp. distribution worldwide (www.discoverlife.org/mp/20m?kind=Gambusia+affinis). The website says it is *G. affinis*, but the dots given in the EU are not valid except for Italy (Carella et al. 2013). Those 'invalid'sites can be assumed to be *G. holbrooki* until genetic evidence is available to disprove this assumption. Note that such maps are for 'illustrative purposes' only and variations between maps and databases (e.g.

		FishBase) can be identified if closely scrutinised.  Figure 2_Q5: Approximate (i.e. incomplete) map of *Gambusia holbrooki* distribution in Europe, with the lone, confirmed established population of *G. affinis* (near Naples, Italy) indicated with a blue arrow. Map from: www.ittiofauna.org/webmuseum/pesciossei/cyprinodontes/poecilidae/gambusia/gambusiaaffinis/gambusiaaffinis.htm
6. Is the organism known to be invasive (i.e. to threaten organisms, habitats or ecosystems) anywhere in the world?	Yes	The aggressive and predatory behaviour of mosquitofish is considered to have adverse impacts on populations of small fish through predation and competition. Introduced mosquitofish reportedly displaced select native fish species in some

		locations, the native being regarded as more effective at controlling mosquitoes (the above information from a review by Nico et al. 2017). Greater details regarding the risks and impacts posed by *G. affinis* are given in Section B and therefore are not repeated here.
7. Describe any known socio-economic benefits of the organism in the risk assessment area.	Despite the species common name, and the global introductions of mosquitofish as mosquito-control agents, reviews of available literature worldwide on mosquito control reveal limited if any evidence that *G. affinis* is effective in reducing mosquito population densities or in reducing the incidence of mosquito-borne diseases (Courtenay and Meffe 1989; Arthington and Lloyd 1989; cited by Nico et al. 2017), including concerns in the 1990s about the adverse environmental impacts of introducing an exotic species into non-native areas (Rupp 1996).	Studies have shown mosquitofishes not to be particularly effective, despite their common names, at controlling mosquitoes, and in fact can benefit mosquitos by relieving competitive pressure from zooplankton and reducing the pressure exerted by predatory invertebrates. Rowe et al. (2008) examined the costs and benefits of the close congener, *G. holbrooki*, and concluded that "the introduction of *Gambusia* has not generated additional economic benefits, which other native fish were not already able to deliver. Therefore the benefits of mosquito control by *Gambusia* are likely to be negligible or at least comparable to other native species. Contrary to redfin perch [i.e. Eurasian perch *Perca fluviatilis*], *Gambusia* do not generate any value for recreational fishers and therefore recreational benefits are likely to be negligible." This said, *Gambusia* species are used as bait (Fritschie & Olden 2016) and forage fish (N. Poulet, personal communication), so there must be some benefit." As such, the potential socio-economic benefits of *G. affinis* are the subject of debate, with potential benefits to society probably limited to a few locations where mosquito larvae are the main (or only) food available to the species and native fishes or amphibians are not affected. However, such benefits appear to be refuted by the review

		papers cited in the response (here to the left), and at least one study has found *Gambusia affinis* to suffer elevated mortality rates, poor growth and delayed maturation when their diet was restricted to mosquito larvae (Reddy & Pandian 1972).

SECTION B – Detailed assessment			
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]**	**COMMENTS**
1.1. How many active pathways are relevant to the potential entry of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	**few**	**medium**	
1.2. List relevant pathways through which the organism could enter. Where possible give detail about the specific origins and end points of the pathways as well as a description of the 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).	A) **RELEASE IN NATURE**: “Biological control” B) **ESCAPE FROM CONFINEMENT** (“Pet/aquarium/terrarium species”) C) **TRANSPORT –**		The unintentional transport of fish encompasses the species being a stowaway, i.e. an organism that is hidden within an intended consignment.

	CONTAMINANT : Contaminant on animals		
Pathway name:	**A) RELEASE IN NATURE** "Biological control"		
1.3a. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	**intentional**	**high**	*Gambusia affinis* may be imported as a biological control agent for mosquito larvae. This already happened in the past in Europe and elsewhere in the world for species of the genus *Gambusia* (see www.fishbase.org and cabi/isc).
1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Sub-note: In your comment discuss how likely the organism is to get onto the pathway in the first place. Sub-note: In your comment discuss the volume of movement along this pathway.	**moderately likely**	**medium**	Intentional import of *G. affinis* for mosquito control in Europe would nowadays be regulated, either under national legislation or the EU Regulation on the use of alien species in aquaculture (which includes stocking). And since there is limited if any evidence that *G. affinis* is effective in reducing mosquito population densities, such importations are not likely to be allowed and/or executed, especially since there are concerns about the adverse environmental impacts of introducing exotic species into non-native areas (Rupp 1996). This assumption of no further importation assumes that MSs of the EU will implement and enforce the EU Regulation on the use of alien species in aquaculture. The limited efficacy of *G. affinis* in controlling mosquitoes is not common knowledge (i.e. to the general public), so it is moderately likely that *Gambusia* could be released into the wild, e.g. garden ponds, throughout the EU where mosquitoes are a problem.
1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the	very unlikely unlikely moderately likely likely very likely	low medium high very high	The template does not require an answer for pathways of intentional entry.

organism could multiply along the pathway.	NA		
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	very unlikely unlikely moderately likely likely very likely NA	low medium high very high	The template does not require an answer for pathways of intentional entry.
1.7a. How likely is the organism to enter Europe undetected?	very unlikely unlikely moderately likely likely very likely NA	low medium high very high	The template does not require an answer for pathways of intentional entry.
1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very unlikely unlikely moderately likely likely very likely NA	low medium high very high	The template does not require an answer for pathways of intentional entry.
1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**very likely**	**high**	If *G. affinis* were to be intentionally introduced for mosquito control, then they probably would be introduced into suitable habitat only.
1.10a. Estimate the overall likelihood of entry into Europe based on this pathway?	**moderately likely**	**high**	See response to Q1.4a.
Pathway name:	**B) ESCAPE FROM CONFINEMENT** (“Pet/aquarium/terrarium species”)		
1.3b. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	**unintentional**	**high**	Used as live food for carnivorous aquarium fishes (Fishbase) and as an ornamental fish (CABI/ISC). They are used in the commercial aquarium industry (www.fishbase.org) but poor sales are likely given their noxious status in many countries, aggressive behaviour, and poor appearance (CABI/ISC).
1.4b. How likely is it that large numbers of the organism	**unlikely**	**medium**	*G. affinis* is available for sale on several N. American

will travel along this pathway from the point(s) of origin over the course of one year? Sub-note: In your comment discuss how likely the organism is to get onto the pathway in the first place. Sub-note: In your comment discuss the volume of movement along this pathway.			websites but a Google search did not reveal any European websites (in English). Transport of live mosquitofish from N. America to Europe for aquarium trade has been reported in low frequency (Maceda-Veiga et al 2013). Existing literature is for *Gambusia* in general, and for *G. holbrooki* (Maceda-Veiga et al. 2013), with none specific to *G. affinis* on this pathway. Based on information in Maceda-Veiga et al. (2013), propagule pressure from this pathway is likely to be very low. However, with the expansion of Asian tiger mosquito *Aedes albopictus* (*Stegomyia albopicta*) into Europe (see e.g. www.ladepeche.fr/article/2018/05/17/2799475-moustique-tigre-mefiez-vous-de-l-eau-qui-dort.html), the risk of further *Gambusia* sp. importations in large numbers is at least moderate, especially as mosquitofishes continue to be sold in some EU countries, e.g. France, http://tortues-terrestres.forumactif.com/t54457-gambusie-sur-perpignan (N. Poulet, personal communication).
1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	**likely**	**high**	If live transport of *G. affinis* were to be organised, then survival during the passage would be high as with other fish transports. Moreover, *G. affinis* is a hardy fish that can withstand reduced dissolved oxygen conditions (Pyke, 2008). Reproduction during the transport is very unlikely.
1.6b. How likely is the organism to survive existing management practices during passage along the pathway?	**moderately likely**	**medium**	The efficacy of the management action will depend upon a variety of factors. The fish could be easily killed using a piscicide, $Ca(OH)_2$ (Lynch, 2008) or desiccation in small and/or large shallow water bodies (e.g. drained marshlands). Whereas, successful control in, or eradication from, larger water bodies, or those encumbered by ligneous and other debris, and in water courses (stream-dwelling *Gambusia* populations are

			known to exist: e.g. Wach & Chambers 2007), especially complex hydrosystems with multiple channels (e.g. flood plains) and connecting canals, would be more difficult.
1.7b. How likely is the organism to enter Europe undetected?	**moderately likely**	**medium**	*G. affinis* is easily confused, due to physical similarity, with other *Gambusia* species, guppies and killifishes.
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**likely**	**high**	If live transport of *G. affinis* were to be organised, then they could happen any time of year.
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**moderately likely**	**medium**	Although suitable habitat is likely to exist, escape from aquarium confinement is unlikely without some form of human assistance but escape from garden pond or similar confinement is moderately likely, especially where those habitats are within a flood plain.
1.10b. Estimate the overall likelihood of entry into Europe based on this pathway?	**moderately likely**	**medium**	Entry in live form for aquarium/ornamental purposes is likely, with escape both unlikely (from aquaria) and likely (from outdoor ponds, especially those within a flood plain).
Pathway name:	**C) TRANSPORT – CONTAMINANT:** Contaminant on animals		
1.3c. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	**unintentional**	**high**	*G. affinis* can easily be a contaminant at imports of the other species for various uses, including mosquito control, pet fish (aquaria/garden ponds) or even other small fish species used for angling (live bait). For example, the guppy *Poecilia reticulata* is a popular aquarium fish of similar appearance to *G. affinis*, so consignments of guppies could have contaminant *G. affinis* in them if the supplier of these guppies also rears *Gambusia*. This phenomenon is already documented for the appearance in the UK of fish species that were contaminants of consignments of intended ornamental fish species, e.g. white sucker *Catostomus commersonii* (Copp et al. 1993) and Asian weatherfish *Misgurnus mizolepis* (Zięba et al. 2010).

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? Sub-note: In your comment discuss how likely the organism is to get onto the pathway in the first place. Sub-note: In your comment discuss the volume of movement along this pathway.	**likely**	**high**	This pathway was responsible for the introduction of two of the most successful invasive fish species in Europe i.e. topmouth gudgeon *Pseudorasbora parva* (Gozlan et al. 2010) and Amur sleeper *Perccottus glenii* (Reshetnikov 2004). Especially overland transport of large containers with live fish for e.g. restocking is likely to be a major pathway of introduction of unwanted species since contaminants in these containers are very difficult to detect. In this way, many thousands of non-wanted specimens can be introduced. Also, *Gambusia* species can be easily mis-identified as a guppy species by anglers (N. Poulet, personal communication)
1.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	**very likely**	**high**	If live transport were to be organised, then survival of the unwanted species e.g. *G. affinis* during the passage would be high as with the other fish species in the transport. Moreover, *G. affinis* is a hardy fish that can withstand reduced dissolved oxygen conditions (Pyke, 2008). Reproduction during the transport is very unlikely.
1.6c. How likely is the organism to survive existing management practices during passage along the pathway?	**likely**	**medium**	Lethal methods cannot be used to remove stowaways from a fish transport. Therefore individual removal of all unwanted specimens is the only possible management practice. This is very difficult and very inefficient and thus it is very likely that several specimens survive this management practice.
1.7c. How likely is the organism to enter Europe undetected?	**likely**	**high**	See response to Q1.4c.
1.8c. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**likely**	**high**	Live transport of fish species (with possible stowaways) can happen any time of year.

1.9c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**likely**	**high**	Often the fish species in the container are imported for restocking and angling purposes. Contaminants in this container will be equally transferred to the receiving waters which often will be suitable habitat for the stowaway species.
1.10c. Estimate the overall likelihood of entry into Europe based on this pathway?	**likely**	**high**	Since this pathway was responsible for the introduction of several successful invasive fish species in Europe (e.g. topmouth gudgeon and Amur sleeper) it is likely that this also can be the case for *G. affinis*.
End of pathway assessments			
1.11. Estimate the overall likelihood of entry into Europe based on all pathways in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	**moderately likely**	**high**	Intentional import of *G. affinis* for pest control seem unlikely nowadays because effectiveness of control is probably limited and moreover there are concerns about the adverse environmental impacts of introducing exotic species into non-native areas (Rupp 1996). The highest risk of introduction into the EU is as a contaminant in transport of live fish from countries outside the EU where *G. affinis* is established (e.g. Turkey).
1.12. Estimate the overall likelihood of entry into Europe based on all pathways in relevant biogeographical regions in foreseeable climate change conditions?	**moderately likely**	**high**	See response to Q1.11 – no change is expected in the near future.

PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the Union, 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 1.15.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.12. How likely is it that the organism will be able to establish in the EU based on the similarity between climatic conditions in Europe and the organism's current distribution?	**very likely**	**high**	*G. affinis* is an adaptable species, and relative to *G. holbrooki*. *G. affinis* is reported to demonstrate equal, if not greater, adaptability in terms of temperature, salinity, etc. (Vidal et al. 2010). *G. holbrooki* is relatively widespread in Europe while *G. affinis* has an established population in Italy (Vidal et al. 2010) confirmed for one location (Carella et al. 2013) only.
1.13. How likely is it that the organism will be able to establish in the EU based on the similarity between other abiotic conditions in Europe and the organism's current distribution?	**very likely**	**high**	See comments to Q1.12 here above.
1.14. How likely is it that the organism will become established in protected conditions (in which the environment is artificially maintained, such as wildlife parks, glasshouses, aquaculture facilities, terraria, zoological gardens) in Europe? Sub-note: gardens are not considered protected conditions	**very likely**	**high**	*G. affinis* is known to prefer lentic, vegetated water bodies with silty bottoms, and the species is known to benefit from disturbance (Lloyd et al. 1986). The protected site examples given with the question are therefore very likely to be ideal for this species.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in Europe?	**moderately widespread**	**high**	Lentic, silt-bottom fresh- and brackish-water ecosystems, inland and coastal, are abundant in the EU, and in view of the cold-tolerant adaptive ability of *G. affinis* (e.g. Hass et al. 2003; Srean 2015), this species could establish in a large part of

			Europe. See as well EU Chapeau responses.
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 Europe?	**NA**	**very high**	This species is not known to depend on other species during critical stages of its life cycle.
1.17. How likely is it that establishment will occur despite competition from existing species in Europe?	**likely**	**medium**	*G. affinis* has already demonstrated the ability to establish in Mediterranean countries, with its close relative *G. holbrooki* demonstrating the ability to out-compete native fishes (Rincón et al. 2002; Caiola & de Sostoa 2005; Alcaraz et al. 2008)
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in Europe?	**likely**	**medium**	Benejam et al. (2009) indicates parasite load decreases with increasing latitude, suggesting greater risk of establishment from southern France northward.
1.19. How likely is the organism to establish despite existing management practices in Europe?	**likely**	**high**	Given the successful establishment of *G. holbrooki* under existing management practices, *G. affinis,* is likely to establish in a similar manner if introduced as widely as *G. holbrooki*.
1.20. How likely are existing management practices in Europe to facilitate establishment?	**likely**	**medium**	Ponds are often subject to disproportionately high siltation rates, and the regulation of lowland water courses (use of weirs and dams) results in ponding. These types of lentic environment, including ditches and canals, are suitable to, and even preferred by *G. affinis*, which also do well in disturbed water bodies (e.g. Lloyd et al. 1986; Pyke 2005). Murphy et al. (2015), however, reported that habitat rehabilitation efforts or modifications to human-built features seem unlikely to affect mosquitofish distribution patterns in Europe. They find that natural abiotic factors are far more important here.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in Europe?	**likely**	**high**	In addition to their tolerance of a wide range of physical properties, *G. affinis* are also, relative to other fishes, highly resistant to the effects of toxins and adverse conditions. They are able to survive

			with little mortality rotenone concentrations of ≈0.5 ppm, which would kill most other fishes. Based on available evidence (reviewed in Pyke 2000), *G. affinis* is only about half as sensitive to rotenone as some species, but it is twice as sensitive than others such as the black mudfish *Neochanna diversus* (Willis & Ling 2000). *G. affinis* is also tolerant of several organophosphate insecticides (Johnson 1978). *Gambusia* spp. can also survive exposure to a voltage gradient of 16 $V \cdot cm^{-1}$, which is lethal to half the individuals of most other species. Female *Gambusia* spp. can also accumulate high concentrations of selenium in their body tissues and still maintain a very high new-born survival rate (summarised from Pyke 2000).
1.22. How likely are the biological characteristics of the organism to facilitate its establishment?	**very likely**	**high**	*G. affinis* is a live-bearing species, which means young of the year are born as 'small juveniles' possessing the capacities, if not the size, of the definitive phenotype (i.e. adult form). The species' fecundity (and other life-history traits) are quite plastic, thus facilitating establishment. For example, fecundity (i.e. brood size) of individual females increases linearly with length and weight, and is affected by the water body's trophic status, with maximum brood size varying greatly, even achieving 428 young. However, mean fecundity ranges 30–50 young (summarised from Lloyd et al. 1986). Males and females of *G. affinis* generally mature at the same size and age. Age and size at maturity decrease in warmer waters. Growth is influenced by food quantity and composition, temperature and salinity, though somatic growth rate was observed

			to peak at intermediate food levels, whereas brood mass and fecundity continue to increase with increasing food supply. That said, *Gambusia* growth and development rates are generally density dependent, decreasing with increasing *Gambusia* density (summarised from Lloyd et al. 1986 and Pyke 2005). This adaptability to local environmental conditions is characteristic of successful fish invaders.
1.23. How likely is the capacity to spread of the organism to facilitate its establishment?	**unlikely**	**high**	This small-bodied species is generally believed to be poorly adapted to lotic waters, but stream-dwelling populations exist, including in the introduced range (e.g. Wach & Chambers 2007; Murphy et al. 2015). *G. affinis* are said to be 'poor dispersers' (Zogaris 2014, citing Pyke 2005), so dispersal from a point source will depend on other, external factors (e.g. humans, birds, contaminant on angling gear) and its connectivity with other water bodies. None of these factors, however, has been demonstrated to facilitate the establishment of self-sustaining populations by *G. affinis*.
1.24. How likely is the adaptability of the organism to facilitate its establishment?	**very likely**	**high**	See comments to Q1.22.
1.25. How likely is it that the organism could establish despite low genetic diversity in the founder population?	**likely**	**low**	No information is available on the genetic diversity of founder *G. affinis* populations in Europe. A study of the effect of a severe bottleneck on genetic variability in four populations of its close congener, *G. holbrooki*, introduced to Spain and Italy in the early 1900s, revealed a strong reduction of genetic diversity relative to a native population in North America. But despite this reduced diversity, *G. holbrooki* has successfully invaded European inland waters (Grapputo et al. 2006). Sanz et al. (2013) found

			that despite evidence of recent bottleneck events in a few isolated locations in Europe, most introduced populations of *G. holbrooki* possessed considerable gene diversity, probably due to multiple introductions and secondary contacts.
1.26. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in Europe? (If possible, specify the instances in the comments box.)	**very likely**	**very high**	*G. affinis* is known globally to be an invasive species (Lowe et al. 2000; Srean 2015; Turbelin et al. 2017).
1.27. If the organism does not establish, then how likely is it that casual populations will continue to occur? Sub-note: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.	**moderately likely**	**low**	Casual populations could persist in suitable water bodies (of which there are many in the EU) if introduction/release takes place.
1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).	**likely**	**high**	It is overall highly likely that the species can establish in the Mediterranean (already established there), Continental and Atlantic biogeographic regions. Most of the western EU falls within the 'Cfb' Köppen-Geiger climate zone (Peel et al. 2007). This extends from northern Spain to southern Denmark and Sweden, encompassing all of the British Isles towards the east to the longitudinal mid-point of Germany, with pockets of Cfb zone in northern Italy, in Slovenia, Croatia, Bosnia Herzogovina, Serbia and Bulgaria, Austria, the Czech Republic and even a thin strip of southern Norway. So, given that the native and introduced ranges of *G. affinis* in the USA (Nico et al. 2017) encompasses even colder and warmer/dryer climate zones (Peel et al. 2007) than Cfb, one can assume that *G. affinis* could establish in most of western and southern EU MSs. The

			adaptive tolerance to colder temperatures of G. affinis (Otto 1973) indicates potential establishment across a broad area of southern to northerly parts of the EU.
1.29. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions	**very likely**	**very high**	Despite its adaptive tolerance to colder temperatures, *G. affinis* prefers warmer water temperatures, so any increase in ambient mean temperatures will benefit this species (Lloyd et al. 1986; Pyke 2005).

PROBABILITY OF SPREAD			
Important notes: • Spread is defined as the expansion of the geographical distribution of an alien species within the assessment area.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENT**
2.1. How important is the expected spread of this organism in Europe by natural means? (Please list and comment on each of the mechanisms for natural spread.)	**minimal**	**medium**	*G. affinis* is a non-migratory species, and the movement of individuals is usually localised, limited to relatively small areas, with some individuals occasionally dispersing over longer distances (Pyke, 2008). Dispersal among river basin depends on human (or avian) intervention, although there is unproven suspicion that pregnant females might be transported by waterfowl – an assumption/suspicion held for the spread of some other fish species, though evidence for it is lacking (see also the Comment at Q2.4c).
2.2. How important is the expected spread of this organism in Europe by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.	**moderate**	**medium**	Initial and subsequent introductions of *Gambusia* species in Europe have been by humans, e.g. for mosquito control (Lloyd et al. 1986; Pyke 2005), with possible use as live bait, such as reported in the USA (Fritschie & Olden 2016), in areas where *Gambusia* species are abundant.
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).	A) **RELEASE IN NATURE**: "Biological control" B) **ESCAPE FROM CONFINEMENT** ("Pet/aquarium/terrarium species") C) **TRANSPORT -**		

	CONTAMINANT: Contaminant on animals D) **UNAIDED**: Natural dispersal		
Pathway name:	**A) RELEASE IN NATURE** "Biological control"		
2.3a. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	**intentional**	**high**	
2.4a. How likely is it that large numbers of the organism will spread along this pathway from the point(s) of origin over the course of one year?	**moderately likely**	**medium**	Intentional importation of *G. affinis* for mosquito control in the EU would nowadays be regulated, either under national legislation or the EU Regulation on the use of alien species in aquaculture (which includes stocking). And since there is limited, if any, evidence that *G. affinis* are particularly effective at reducing the density of mosquito populations, such importations are not likely to be allowed and/or executed, especially since there are concerns about the adverse environmental impacts of introducing exotic species into non-native areas (Rupp 1996). This assumption of no further importation assumes that MSs of the EU will implement and enforce the new EU Regulation mentioned here above. The limited efficacy of *G. affinis* in controlling mosquitoes is not common knowledge (i.e. to the general public), so it is moderately likely that this species could be released into the wild, e.g. garden ponds, throughout the EU where mosquitoes are a problem. However, with the advancement in parts of Europe of the Asian tiger mosquito *Aedes albopictus* (*Stegomyia albopicta*)(www.ladepeche.fr/article/2018/05/17/2799475-moustique-tigre-mefiez-vous-de-l-eau-qui-dort.html),

			the risk of further *G. affinis* introductions is at least moderate, especially as mosquitofishes continue to be sold, e.g. France, e.g. http://tortues-terrestres.forumactif.com/t54457-gambusie-sur-perpignan (N. Poulet, personal communication).
2.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	**likely**	**high**	If *G. affinis* would be imported for biological control, then their survival would be high when transport is well organised. Reproduction is not expected to happen during this passage unless "pregnant" females are present in the transport, which can then "give birth" to many individuals.. Also, the species is relatively robust with regard to poor water quality (e.g. during transport).
2.6a. How likely is the organism to survive existing management practices during spread?	**moderately likely**	**medium**	Although several management practices to extirpate *G. affinis* exist, e.g. piscicide, Ca $(OH)_2$ (Lynch, 2008) or desiccation, most of them would be difficult to apply in rivers, brooks and ponds with native fish and other species present. Eradication of small-bodied fishes is possible (e.g. Britton et al. 2008), and these can be implemented in smaller water bodies with mitigated collateral damage to native species, but legal and management options currently vary across the EU, which increases the overall likelihood of survival under existing management practices.
2.7a. How likely is the organism to spread in Europe undetected?	**moderately likely**	**medium**	Although many MSs have fish monitoring programmes, it could take several years before *G. affinis* was noticed, depending upon the monitoring systems and public awareness at the national, regional and local levels.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	**moderately likely**	**medium**	Responses in the EU Chapeau section indicate a relatively large area of the EU as being suitable to *G. affinis*, especially given the extensive network of water bodies and the increasingly lentic character of regulated water courses.
2.9a. Estimate the overall likelihood of spread into or	**moderately likely**	**medium**	The scientific results that demonstrate the limited

within the Union based on this pathway?			effectiveness of *G. affinis* in controlling mosquitoes is not common knowledge (i.e. to the general public), so it is moderately likely that this species could be released into the wild, e.g. garden ponds, throughout the EU where mosquitoes are a problem. Where such ponds are located within a flood plain, the risk of wider dispersal is enhanced.
Pathway name:	**B) ESCAPE FROM CONFINEMENT** ("Pet/aquarium/terrarium species")		
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**	
2.4b. How likely is it that large numbers of the organism will spread along this pathway from the point(s) of origin over the course of one year?	**moderately likely**	**medium**	Transport of live mosquitofish from N. America to Europe for aquarium trade has been reported in low frequency (Maceda-Veiga et al 2013). However, there is no specific literature for *G. affinis* on this pathway. Based on information in Maceda-Veiga et al. (2013), propagule pressure from this pathway is likely to be very low for mosquitofishes. However, unwanted pet fish can be released to the wild if the owner does not wish to kill the animal to get rid of it (Copp et al. 2005), and if *G. affinis* are released into a garden pond situated in a flood plain, then the potential for wider dispersal is enhanced (i.e. the pond ceases to be 'confinement'). Note that *G. affinis* are sold on several N. American websites, but a Google search did not reveal any European websites (in English) selling *G. affinis*. That said, Maceda et al. (2013) found evidence for a low frequency of importation of *Gambusia* from North America to Europe.
2.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)?	**moderately likely**	**high**	*G. affinis* are robust with regard to water quality, so any escape from confinement via an aquatic means is likely to be successful. Reproduction along the

Sub-note: In your comment consider whether the organism could multiply along the pathway.			pathway (during dispersal) is perhaps possible but seems unlikely, at least not before the fish reach their new habitat.
2.6b. How likely is the organism to survive existing management practices during spread?	**moderately likely**	**high**	Although several management practices to extirpate *G. affinis* exist, e.g. piscicide, $Ca(OH)_2$ (Lynch, 2008) or desiccation, most of them would be difficult to apply in rivers, brooks and ponds with native fish and other species present. Eradication of small-bodied fishes is possible (e.g. Britton et al. 2008), and these can be implemented in smaller water bodies with mitigated collateral damage to native species, but legal and management options currently vary across the EU, which increases the overall likelihood of survival under existing management practices.
2.7b. How likely is the organism to spread in Europe undetected?	**moderately likely**	**high**	Although many MS have fish monitoring programmes, it could take several years before *G. affinis* would be noticed, especially as the locations where abandoned pet fish are released are less likely to be part of a monitoring programme (i.e. small water bodies are not considered under the Water Framework Directive).
2.8b. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	**likely**	**high**	The release of fish by pet owners, and the dispersal of fish during floods, e.g. from one water body to another, is likely (e.g. Copp et al. 2005; Fobert et al. 2013).
2.9b. Estimate the overall likelihood of spread into or within the Union based on this pathway?	**moderately likely**	**medium**	The propensity of pet owners to release fish is a worldwide phenomenon, in some cases the fish are unwanted pets, in some cases the release is part of a religious practice (reviewed in Copp et al. 2005).
Pathway name:	**C) TRANSPORT - CONTAMINANT:** Contaminant on animals		
2.3c. Is spread along this pathway intentional (e.g. the organism is released at distant localities) or unintentional (the organism is a contaminant of imported goods)?	**intentional**	**high**	*G. affinis* can be inadvertently transferred to new water bodies as a contaminant of intentional fish transfers for purposes of re-stocking angling fisheries,

			etc. (N. Poulet, personal communication).
2.4c. How likely is it that large numbers of the organism will spread along this pathway from the point(s) of origin over the course of one year?	**unlikely**	**low**	This pathway was responsible for the introduction of two of the most successful invasive fish species in Europe i.e. topmouth gudgeon *Pseudorasbora parva* (Gozlan et al. 2010) and Amur sleeper *Perccottus glenii* (Reshetnikov 2004). Given that only one population is known to be established in the EU, the chances are small that the general public and anglers will transfer this fish species for use as bait, to enhance fisheries, to dispose of unwanted pets, as part of religious practices, etc.
2.5c. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	**likely**	**high**	If live transport were to be organised, then survival of the unwanted species e.g. *G. affinis* during the passage would be high as with the other fish species in the transport. Moreover, *G. affinis* is a hardy species that can withstand reduced dissolved oxygen conditions (Pyke 2008). Reproduction during the transport is very unlikely.
2.6c. How likely is the organism to survive existing management practices during spread?	**moderately likely**	**medium**	Although several management practices to kill *G. affinis* exist, e.g. piscicide, Ca $(OH)_2$ (Lynch, 2008) or desiccation, most of them would be difficult to apply in rivers, brooks and ponds with native fish and other species present. Eradication of small-bodied fishes is possible (e.g. Britton et al. 2008), and these can be implemented in smaller water bodies with mitigated collateral damage to native species, but legal and management options currently vary across the EU, which increases the overall likelihood of survival under existing management practices.
2.7c. How likely is the organism to spread in Europe undetected?	**moderately likely**	**medium**	Although many MS have fish monitoring programmes, it could take several years before *G. affinis* was noticed, especially as the locations where abandoned pet fish are released are less likely to be part of a monitoring programme (i.e. small water bodies are not considered under the Water Framework

			Directive).
2.8c. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	**moderately likely**	**medium**	Often the fish species in the container are imported for restocking and angling purposes. Contaminant in this container will be equally transferred to the receiving waters which often will be suitable habitat for the stowaway species.
2.9c. Estimate the overall likelihood of spread into or within the Union based on this pathway?	**moderately likely**	**high**	Overland transport of large containers with live fish for e.g. restocking is likely to be a major pathway of introduction of unwanted species since contaminants in these containers are very difficult to detect. Every year, large numbers of fish are transported this way. In this way, many thousands of non-wanted specimens can be introduced over a large number of widespread sites.
Pathway name:	**D) UNAIDED – Natural dispersal**		
2.3d. 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**	*G. affinis* can disperse from infested ponds through ditches in riverine systems. *G. affinis* is robust with regard to water quality, so any dispersal via an aquatic means is likely to be successful.
2.4d. How likely is it that large numbers of the organism will spread along this pathway from the point(s) of origin over the course of one year?	**moderately likely**	**medium**	*G. affinis* is non-migratory, and the movement of individuals is usually localised, limited to relatively small areas, with some individuals occasionally dispersing over longer distances (Pyke, 2008).
2.5d. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Sub-note: In your comment consider whether the organism could multiply along the pathway.	**likely**	**high**	The survival of *G. affinis* during the passage would be high as they are hardy fish that can withstand a.o. reduced dissolved oxygen conditions (Pyke, 2008). Reproduction during the transport is unlikely but not impossible.
2.6d. How likely is the organism to survive existing management practices during spread?	**moderately likely**	**medium**	Although several management practices to kill *G. affinis* exist, e.g. piscicide, $Ca(OH)_2$ (Lynch, 2008) or desiccation, most of them would be difficult to apply

			in rivers, brooks and ponds with native fish and other species present. Eradication of small-bodied fishes is possible (e.g. Britton et al. 2008), and these can be implemented in smaller water bodies with mitigated collateral damage to native species, but legal and management options currently vary across the EU, which increases the overall likelihood of survival under existing management practices.
2.7d. How likely is the organism to spread in Europe undetected?	**moderately likely**	**medium**	Although many MS have fish monitoring programmes, it could take several years before *G. affinis* was noticed, especially as the locations with optimal habitat are less likely to be part of a monitoring programme (i.e. small water bodies are not considered under the Water Framework Directive).
2.8d. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	**moderately likely**	**medium**	As unaided spread would mainly start from infested ponds there would possibly be receiving waters with suitable habitat in the neighbourhood.
2.9d. Estimate the overall likelihood of spread into or within the Union based on this pathway?	**likely**	**medium**	As known from other small-bodied fish species e.g. topmouth gudgeon, the likelihood of spread through this pathway would be likely, especially in the southern parts of the EU.
End of pathway assessments.			
2.10 (formerly 2.3). Within Europe, how difficult would it be to contain the organism?	**difficult**	**high**	Since the source population of *G. affinis* in the EU is very small and possibly limited to a single pond in Italy, it is unlikely that large numbers of specimens of this species will spread within the EU. However, since the likeliest possible pathway, i.e. as a contaminant in live fish transport, is very difficult to control, containment is likely to be difficult.
2.11 (formerly 2.4). Based on the answers to questions on the potential for establishment and spread in Europe, define the area endangered by the organism.	Based on a high potential for establishment and a moderate spread	**medium**	See responses to Qs EUc-3 and EUc-4 in the EU Chapeau section.

	potential; the EU area endangered the most is the Mediterranean, but more northerly locations are also at risk due to the species' greater tolerance to (and persistence in) areas subject to ≤0°C winter temperatures.		
2.12 (formerly 2.5). What proportion (%) of the area/habitat suitable for establishment (i.e. those parts of Europe were the species could establish), if any, has already been colonised by the organism?	**< 1**	**high**	*G. affinis* in the EU is currently considered limited to a single pond in Italy,
2.13 (formerly 2.6). What proportion (%) of the area/habitat suitable for establishment, if any, do you expect to have been invaded by the organism five years from now (including any current presence)?	**1-10**	**low**	If no new introductions and releases take place, the invaded area should remain low.
2.14 (formerly 2.7). What other timeframe (in years) would be appropriate to estimate any significant further spread of the organism in Europe? (Please comment on why this timeframe is chosen.)	**20**	**low**	The potential for spread of *G. affinis* is expected to remain low, but with regulations put into place to control the sale and movement of *G. affinis*, their spread should be expected to decrease over a 20-year time frame.
2.15 (formerly 2.8). In this timeframe what proportion (%) of the endangered area/habitat (including any currently occupied areas/habitats) is likely to have been invaded by this organism?	**1–10**	**low**	If no new introductions and releases take place, the invaded area should remain low.
2.16 (formerly 2.9). Estimate the overall potential for spread in relevant biogeographical regions under current conditions for this organism in Europe (using the comment box to indicate any key issues).	**slowly**	**low**	*G. affinis* is said to be a poor dispersers (Zogaris 2014, citing Pyke 2005), so spread is very likely to be slow under current conditions although human aid may speed up spread in relevant biogeographical

			regions. The human aided mechanisms are, however, very hard to predict.
2.17 (formerly 2.10). Estimate the overall potential for spread in relevant biogeographical regions in foreseeable climate change conditions	**slowly**	**low**	*G. affinis* prefers warmer water temperatures, so any increase in ambient mean temperatures will benefit this species (Lloyd et al. 1986; Pyke 2005). Although establishment is expected to be easier in larger parts of the EU, still spread is very likely to be slow. Nevertheless, human aid may speed up.

MAGNITUDE OF IMPACT			
Important instructions: • When assessing potential future impacts, climate change should not be taken into account. This is done in later questions at the end of the assessment. • Where one type of impact may affect another (e.g. disease may also cause economic impact) the assessor should try to separate the effects (e.g. in this case note the economic impact of disease in the response and comments of the disease question, but do not include them in the economic section). • Note questions 2.18–2.24 to environmental impact, 2.25–2.29 relate to economic impact, and 2.30–2.35 to social impact. Each set of questions starts with the impact elsewhere in the world, then considers impacts in Europe separating known impacts to date (i.e. past and current impacts) from potential future impacts.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**
Environmental impacts			
2.18. How important is impact of the organism on biodiversity and related ecosystem services caused by the organism in its non-native range excluding the Union?	**major**	**high**	Evidence of adverse impacts on biodiversity and ecosystem services is available for various locations in the world where *G. affinis* have been introduced (e.g. Lloyd et al. 1986). For example, Rowe et al. (2008) stated that "…*Gambusia* is clearly responsible for a reduction in indigenous fauna including some rare and threatened fish and amphibian species." In contrast to direct impacts (predation, competition) to native fishes, evidence for a disease risk to native species posed by *Gambusia* species appears to be low, given the lack of intermediate hosts (in the introduced range) for the parasites to which *G. affinis* is susceptible (Benejam et al. 2009). This results in a reduced parasite load (i.e. 'enemy escape theory may apply), and these diseases are apparently specific to *Gambusia*, thus assumed to pose little or no risk to native fishes.
2.19. How important is the impact of the organism on biodiversity (e.g. decline in native species, changes in native species communities, hybridisation) and related	**moderate**	**low**	No direct evidence is available for *G. affinis.* Evidence from the Mediterranean biogeographic region, and specifically the Iberian peninsula indicates that small,

ecosystem services currently in the different biogeographical regions or marine sub-regions where the species has established in Europe (include any past impact in your response)?			endemic fish species are endangered (risk of localised extinctions) by introductions/presence of the close congener, eastern mosquitofish *G. holbrooki* (e.g. Caiola & de Sostoa 2005; Silva et al. 2015; Zogaris 2014, 2017).
2.20. How important is the impact of the organism on biodiversity and related ecosystem services likely to be in the future in the different biogeographical regions or marine sub-regions where the species can establish in Europe?	**moderate**	**medium**	In the near future, under current European climate conditions, the likelihood of *G. affinis* exerting adverse impacts on biodiversity and ecosystem services will increase because current climate conditions (e.g. 2018) include exceptionally warm summers, as although adaptable to colder climates, these species do best in warmer climates. This evaluation is based on the existing impacts of *G. holbrooki*, a very close congener of *G. affinis*.
2.21. How important is alteration of ecosystem function (e.g. habitat change, nutrient cycling, trophic interactions), including losses to ecosystem services that build on these functions, caused by the organism currently in the different biogeographical regions or marine sub-regions where the species has established in Europe (include any past impact in your response)?	**moderate**	**medium**	In some systems, *G. affinis* may have the potential to affect other predator populations (Haas & Pal 1984), such as reducing or eliminating early instars of predaceous aquatic insects (Bence 1982; cited in Sutherst 2004). *G. affinis* have also been found to reduce rotifer, crustacean and insect populations, permitting the extraordinary development of phytoplankton blooms. Other impacts include increased turbidity, dissolved organic phosphorus and temperature, decreased dissolved inorganic phosphorus, and inhibition of *Spirogyra* (Hurlbert et al. 1972).
2.22. How important is alteration of ecosystem function (e.g. habitat change, nutrient cycling, trophic interactions), including losses to ecosystem services that build on these functions, caused by the organism likely to be in the different biogeographical regions or marine sub-regions where the species can establish in Europe in the future?	**major**	**medium**	In the event of wider dispersal of *G. affinis* within the EU, the magnitude of wider dispersal being greater for *G. affinis* than its close congener *G. holbrooki*, other Mediterranean endemic fishes would be put at risk, thus potentially reducing native biodiversity and even species extinction. The impacts on ecosystem function, as described in 2.14, are likely to be exacerbated, given

			that *G. affinis* prefer warmer waters, where eutrophication processes are also favoured. The effects on ecosystem services are more difficult to assess due to vast differences in peoples' perceptions (García-Llorente et al. 2008). However, as a general principle, "to secure the generation of ecosystem services from fish populations, management approaches need to address the fact that fish are embedded in ecosystems and that substitutions for declining populations and habitat losses, such as fish stocking and nature reserves, rarely replace losses of all services." (cited from Holmlund & Hammer 1999).
2.23. How important is decline in conservation value (e.g. sites of nature conservation status, WFD classification) caused by the organism currently in Europe?	**major**	**medium**	Although *G. affinis* has been confirmed for only one water body (a pond near Cancello Arnone, Campania, Caserta, Italy), the adverse impact of *G. holbrooki* on endemic species in Iberia, and their likely contribution to eutrophication processes in other waters they have invaded (Hurlbert et al. 1972) suggests a major impact.
2.24. How important is decline in conservation value (e.g. sites of nature conservation status, WFD classification) caused by the organism likely to be in the future in Europe?	**major**	**low**	There is no evidence relating to impacts related to *G. affinis*, so the examples are provided here below for its close congener, *G. holbrooki*. Three fishes endemic to Iberia, the Spanish toothcarp *Aphanius iberus*, Valencia toothcarp *Valencia hispanica*, and Andalusian toothcarp *Aphanius baeticus* are all afforded conservation protection status under the Spanish and IUCN Red lists when research into the impacts of *G. holbrooki* were being undertaken (Rincón et al. 2002; Caiola & de Sostoa 2005; Oliva-Paterna et al. 2006). Caiola & de Sostoa (2005) suggested that these Spanish and Valencia toothcarp species should be reclassified to the highest protection level, and in 2006

			they were reclassified as 'Critically Endangered', complemented by additional classifications under other nature conservation legislation (Silva et al. 2015): •Listed as priority for conservation in Annexes II and IV of the Habitats Directive • Art 17 Conservation status (2007–2012): 'Unfavourable-bad'. In Cyprus, *G. holbrooki* is also believed to threaten the Mediterranean banded killifish *Aphanius fasciatus* (Zogaris 2014, 2017). *Gambusia holbrooki*, has been demonstrated to exert impacts on these two endemic toothcarp species, as well as to the critically-endangered Corfu toothcarp *Valencia letourneuxi*, which is endemic to Western Greece and Southern Albania, and likely to be suffering both competition and predation pressure from *G. holbrooki* (Kalogianni et al. 2014). Given the very strong similarity between these *Gambusia* species, as well as the similarity in their impacts on native fishes where introduced elsewhere in the world (e.g. Nico et al. 2017a, 2017b), it is reasonable to assume that *G. affinis* will exacerbate the impacts on native fishes where *G. holbrooki* has already exists (e.g. displacement; Carmona-Catot et al. 2013) and exert similar adverse impacts on native fauna elsewhere in the suitable parts of the EU where no *Gambusia* species currently exist. To protect these endemic fishes, some nature (biodiversity) reserves have been set up in Iberia (e.g. Parque Natural La Albufera, Spain), following in part to calls for such reserves (e.g. Mata & Núñez de Arenas 2000; Pino-del Carpio et al. 2010; Darwall et al. 2014), to protect biodiversity in general, but including the

			Valencia toothcarp (www.samarucs.org/cas/muntanyisme/actualitat-126-reserves_del_samaruc_valencia_hispanica_.html): These are the natural parks in Valencia region: (www.agroambient.gva.es/web/espacios-protegidos/parques-naturales-2371), which are 'fluvial natural reserves' (www.mapama.gob.es/es/ministerio/patrimonio/Reservas_fluviales.aspx) within the Parque Natural La Albufera. Habitat Directive designations (Red Natura 2000) also exist for protected areas in Spain (of lower status than national and natural parks) that have these two toothcarp species amongst the protected species: (www.mapama.gob.es/es/biodiversidad/temas/espacios-protegidos/red-natura-2000/rn_tip_hab_esp_tipos_habitat_IC.aspx)
Economic impacts			
2.25. How great is the overall economic cost caused by the organism within its current area of distribution, including both costs of damage and the cost of current management	**major**	**low**	Cost estimates regarding *G. affinis* or its close relative *G. holbrooki* could not be found anywhere in Europe. There appear to have been some restoration/rehabilitation/control initiatives that have been funded, at least in the Iberian Peninsula, but details needed to make an estimate of restoration costs are not available. Estimates of control costs for small-bodied alien freshwater fishes within the EU are rare, but one is available for the UK (Britton et al. 2008), where invasive fish eradication costs were found to vary considerable according to local site specifics, but the

			overall estimate is £2 per m^2 (€2.25 per m^2), which is ≈ €22.5k per hectare. The only costs associated are from *G. affinis* close relative *G. holbrooki*. Overall cost estimates come from the USA and Australia. The cost estimates from Australia refers to research and control costs of projects over a five-year period. For *Gambusia holbrooki*, these were estimated in 2003 for New South Wales (NSW) for the NSW threat abatement plan: AU$220k (≈€149k). And funding of the new MDBA project 'Native fish recovery following the removal of alien species', was ≈AU$450k (≈€305k) over three years (Macdonald & Tonkin 2008; Rowe et al. 2008). Half the costs cited in the 2003 NSW abatement plan were attributed to research, the remaining being split between monitoring and control costs such as creating supplementary habitat and chemical control trials (Rowe et al. 2008). These amounts would seem relatively modest, given the following example: The Pobles del Sud lagoon in the Parque Natural La Albufera (Spain) has a surface area of 21,120 hectares (https://en.wikipedia.org/wiki/Albufera) and contains the two endangered toothcarp species (Aleixandre-Roig 2004). Based on the ≈€2.25 per hectare cost (Britton et al. 2008) for eradicating a small-bodied freshwater fish of similar size to *G. affinis*, the estimated cost of eradicating *G. affinis* from that lagoon would be about €475,200, which is over 3× and 1.6× the budgets allocated to the 2003 NSW and MDBA projects, respectively, mentioned for Australia here above. If we compare only the 'monitoring & control' portion mentioned above for the NSW abatement programme

			(50% of the total), then the cost of eradicating *Gambusia* spp. from Pobles de Sud represents ≈ 6× the budget allocated for the species throughout all of the Australian state of NSW, which is about 1.6× the surface area of Spain. The only European estimate that could be found is for Spain, where costs of controlling invasive plants, based on a limited dataset for the 10-year period leading up to 2009, indicated a total actual expenditure €50M (Andreu et al. 2009). So, considering only management costs as a barometer, the overall costs are likely to be at least major, though the confidence level in this is at best moderate.
2.26. How great is the economic cost of damage*of the organism currently in the Union (include any past costs in your response)? *i.e. excluding costs of management	**major**	**low**	Costs specifically for *G. affinis* are difficult to find. However, in general, the cost for alien fishes in the USA per annum, for losses and damages only (i.e. excluding control costs) was estimated initially to be 5.4 billion USD (Pimentel et al. 2005), which is an update of the 1 billion U$D estimate provided in Pimentel et al. (2000). Using the cost estimates from Pimentel et al. (2005) for losses and damage on a per area of water basis, the following estimate can be put forward (data from Wikipedia): USA total area = 9,833,520 km^2 % water = 6.97 area of water = 685,396 km^2 Total losses & damage (Pimentel et al. (2005) = 5.4 billion Losses & damage ‘water only’ = U$D 376.4M (€337M)

			EU total area = 4,475,757 km^2 % water = 3.08 area of water = 137,853 km^2 proportion relative to the USA = 20.113% Losses & damage 'water only' = U$D 75,7M (€68M) As a rough indicator, the overall costs are likely to be at least major, though the confidence level in this is at best moderate.
2.27. How great is the economic cost of damage* the organism likely to be in the future in the Union? *i.e. excluding costs of management	**major**	**low**	No information was found. Indeed, there are no estimates available specifically for *G. affinis* for either current or future periods for the EU. And costs specifically for *Gambusia* species are difficult to find. However, if we assume that not more than 5% of suitable European inland and coastal waters are inhabited by *Gambusia* species, then the rough estimate of future damage would be ≈5% of €68M ≈ €3.4M. This is a very rough estimate for the purposes of this risk assessment, hence 'major' cost but 'low' confidence.
2.28. How great are the economic costs associated with managing this organism currently in the Union (include any past costs in your response)?	**moderate**	**low**	Eradication costs for another small-bodied invasive fish species in Europe, i.e. topmouth gudgeon *Pseudorasbora para*, provide the best available approximation of costs, which at €20.5k per hectare (Britton et al. 2008) suggest moderate management costs at the EU scale. Given all the uncertainty, confidence ranking is 'Low'.
2.29. How great are the economic costs associated with managing this organism likely to be in the future in the Union?	**major**	**low**	Here again, speculation depends on rough estimates. Invasive fish eradication costs have been examined in one EU member state, the UK (Britton et al. 2008) – the

			total costs vary considerable according to local site specifics, but the overall estimate is £2 per m^2 (€2.25 per m^2), which is ≈ €22.5k per hectare. The only confirmed population of *G. affinis* is in Italy (see early Comments), and if we assume that not more than 5% of inland waters of Italy have been invaded by *G. affinis* (i.e. other genetic studies of *Gambusia* in Italy found only *holbrooki*), then the eradication cost for *G. affinis* would be ≈ €741k.
Social impacts			
2.30. How important is social, human health or other impact (not directly included in economic and environmental categories) caused by the organism for the Union and for third countries, if relevant (e.g. with similar eco-climatic conditions).	**minor**	**low**	The majority of information on impacts related to social and human health issues address the safety surrounding the use of biocontrol methods (i.e. *G. affinis* to control mosquitoes) to improve human health (i.e. reduction in mosquito-born diseases) (e.g. Benelli et al. 2016). There is a long-standing debate as to whether or not *G. affinis* actually reduce mosquito numbers (Rupp 1996), with the disadvantages of the adverse impacts on native biodiversity and ecosystem considered to outweigh the potential benefits of *G. affinis* introductions (see responses to Qs 2.18–2.24). As such, potential 'benefits' to society of *G. affinis* introductions may exist, at least in some situations, but these considerations do not contribute to the response given here regarding 'adverse' impacts of *G. affinis* on society and human health. Evidence for a disease risk posed by *G. affinis* appears to be low, given that a lack of intermediate hosts (in the introduced range) for the parasites to which *G. affinis* is susceptible (Benejam et al. 2009). This results in a reduced parasite load (i.e. 'enemy escape theory may

			apply). The one potential adverse impact for human health of *G. affinis* appears to be that their presence favours bilharziasis (Schistosomiasis) vectors (Haas & Pal 1984), the latter being parasitic blood flukes (trematodes) that have free-swimming larvae that penetrate the skin of persons swimming or wading in the water. However, Acra et al. (1986) reported that *G. affinis* preferentially preyed on the snail hosts of this disease, thus reducing the prevalence in a laboratory study. Several other papers were found that referred to the use of *Gambusia* introductions to control *Schistosoma* prevalence. Probably the most apparent adverse impact of *G. affinis* to human health and safety would be its role in contributing to eutrophication (Hurlbert et al. 1972), i.e. poor water quality, which favours the development of water-borne diseases (Pandey et al. 2014).
2.31. How important is social, human health or other impact (not directly included in economic and environmental categories) caused by the organism in the future for the Union.	**moderate**	**low**	Based on the comments to Q2.30, in the near future, under current European climate conditions, the impacts are likely to increase because current climate conditions (e.g. 2018) include exceptionally warm summers, which favour *G. affinis* and eutrophication processes.
2.32. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	**minor**	**low**	See comments to Q2.30.
2.33. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	**NA**	**medium**	Moderately confident that this question is not applicable as no other impacts are recalled.

2.34. 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 Europe?	**moderate**	**low**	See comments to Q2.30 and Q2.31.
2.35. Indicate any parts of Europe where economic, environmental and social impacts are particularly likely to occur (provide as much detail as possible).	**EU countries of the Mediterranean Region**	**medium**	See comments to previous questions in this 'Impact' section.

ADDITIONAL QUESTIONS - CLIMATE CHANGE			
3.1. What aspects of climate change, if any, are most likely to affect the risk assessment for this organism?	Increased (annual mean and winter minimum) water temperature	**very high**	The main limiting factor in the establishment of *G. affinis* is water temperature, in particular during winter. These species are not normally present in high and intermediate elevations (Murphy et al. 2015) in part due to temperature but also water velocities (see also Srean 2015). So if precipitation and water velocities decrease (e.g. due to agricultural use and increased evapo-transpiration), then the distribution of mosquitofishes could be enhanced. In the case of *G. affinis*, which currently is believed to have a very limited distribution, it has the potential to expand to have a distribution in the EU at least as widely as *G. holbrooki*, including into the more northerly countries due to the greater, but less well recognised, cold-tolerance of both species.
3.2. What is the likely timeframe for such changes?	**20** years	**medium**	A 20-year time span is likely to be needed for winter minimum water temperatures to increase to within the near-zero °C minimum reported for *Gambusia* species in recent publications.
3.3. What aspects of the risk assessment are most likely to change as a result of climate change?	Establishment and Impacts	**high**	With increased establishment success and frequency will come increased ecological and other impacts.
ADDITIONAL QUESTIONS - RESEARCH			
4.1. If there is any research that would significantly strengthen confidence in the risk assessment please summarise this here.	1) Ecophysiology 2) Impacts on:	**high**	• The ecophysiological basis of cold adaptation in *G. affinis* Impacts on: • Native species and biodiversity in terms of

	• Biodiversity • Ecosystem function • Ecosystem services • Socio-economic aspects		endemic species, including risks associated with parasite and pathogen transmission. • Aquatic ecosystem function, especially outside of Iberia • Ecosystem services associated with *Gambusia* invasions and management • Social and economic impacts associated with *Gambusia* invasions and management

REFERENCES:

Acra, A., Milki, R., Raffoul, Z., Karahagopian, Y. & Fletcher, M. 1986. Laboratory evaluation of *Gambusia affinis* fish as predators of the schistosome-bearing snails *Bulinus truncatus.* The Journal of tropical medicine and hygiene, 89(1), pp.7-12.

Alcaraz, C., Bisazza, A. & García-Berthou, E., 2008. Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia* 155, 205–213.

Aleixandre Roig, V. 2004. Dunas Albufera - Model of restoration of dunes habitats in 'L'Albufera de Valencia'. LIFE00 NAT/E/007339 (http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=1785)

Almeida Real, D., Ribeiro, F., Leunda, P.A. Vilizzi, L. & Copp, G.H. 2013. Effectiveness of an invasiveness screening tool for non-native freshwater fishes (FISK) to perform risk identification assessments in the Iberian Peninsula. Risk Analysis 33, 1404–1413.

Andreu, J., Vila, M. and Hulme, P.E., 2009. An assessment of stakeholder perceptions and management of noxious alien plants in Spain. Environmental Management 43, 1244.

Arthington, A.H. & Lloyd, L.N. 1989. Introduced poeciliids in Australia and New Zealand. Pages 333–348 in G.K. Meffe and F.F. Snelson, Jr., editors. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, NJ.

Arthur, J.R. and Subasinghe, R.P. 2002. Potential adverse socio-economic and biological impacts of aquatic animal pathogens due to hatchery-based enhancement of inland open-water systems, and possibilities for their minimisation. FAO Fisheries Technical Paper, pp.113–126.

Beaudouin, R., Ginot, V. & Monod, G., 2008. Growth characteristics of eastern mosquitofish G*ambusia holbrooki* in a northern habitat (Brittany, France). Journal of Fish Biology 73, 2468–2484.

Bence, J.R. 1982. Some interactions of predaceous insects and mosquitofish *Gambusia affinis*: a review of some recent results. Bull. Soc. Vector Ecol. 7:41-44.

Benejam, L., Alcaraz, C., Sasal, P., Simon-Levert, G. & García-Berthou, E. 2009. Life history and parasites of the invasive mosquitofish (*Gambusia holbrooki*) along a latitudinal gradient. Biological Invasions 11, 2265–2277.

Benelli, G., Jeffries, C.L. & Walker, T. 2016. Biological control of mosquito vectors: Past, present, and future. Insects 7, 52 (doi:10.3390/insects7040052)

Britton, J.R., Brazier, M., Davies, G.D. & Chare, S.I. 2008. Case studies on eradicating the Asiatic cyprinid topmouth gudgeon *Pseudorasbora parva* from fishing lakes in England to prevent their riverine dispersal. Aquatic Conservation 18, 867–876.

Caiola N. & de Sostoa A. 2005. Possible reasons for the decline of two native toothcarps in the Iberian Peninsula: evidence of competition with the introduced Eastern mosquitofish. Journal of Applied Ichthyology 21, 358–363.

Carella, F., Uliano, E., Esposito, L., Agnisola, C. & De Vico, G., 2013. Presence of *Gambusia affinis* (Baird & Girard, 1853) in a freshwater ecosystem of Campania region (Italy). In VIII Simposium Internacional de Fauna Salvaje (pp. 97–97). Ediccion WAVES.

Carmona-Catot G, Magellan K, García-Berthou E. 2013. Temperature-specific competition between invasive mosquitofish and an endangered Cyprinodontid fish. PLoS ONE 8: e54734.

Copp, G.H., Britton, J.R., Wesley, K.J. & Davison, P.I. 2017. Non-native fish dispersal as a contaminant of aquatic plant consignments – a case study from England. Management of Biological Invasion*s* 8, 437–442.

Copp, G.H., Vaughn, C. & Wheeler, A.C. 1993. First occurrence of the North American white sucker *Catostomus commersoni* in Great Britain. Journal of Fish Biology 42, 615–617.

Copp, G.H., Wesley, K.J. & Vilizzi, L. 2005. Pathways of ornamental and aquarium fish introductions into urban ponds of Epping Forest (London, England): the human vector. Journal of Applied Ichthyology 21, 263–274.

Copp, G.H., Vilizzi, L., Mumford, J.D., Fenwick, G.V., Godard, M.J. & Gozlan, R.E. 2009. Calibration of FISK, an invasive-ness screening tool for non-native freshwater fishes. Risk Analysis 29, 457–467.

Copp, G.H., Vilizzi, L., Tidbury, H., Stebbing, P.D., Tarkan, A.S., Moissec, L. & Goulletquer, Ph. 2016. Development of a generic decision-support tool for identifying potentially invasive aquatic taxa: AS-ISK. Management of Biological Invasions 7, 343–350.

Courtenay, W.R., Jr. & Meffe, G.K. 1989. Small fishes in strange places: a review of introduced poeciliids. Pages 319-331 In: G. K. Meffe, and F. F. Snelson, Jr., editors. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, NJ.

Darwall, W., Carrizo, S., Numa, C., Barrios, V., Freyhof, J. and Smith, K. (2014). *Freshwater Key Biodiversity Areas in the Mediterranean Basin Hotspot: Informing species conservation and development planning in freshwater ecosystems.* Cambridge, UK and Malaga, Spain: IUCN. x + 86pp.

Economou, A.N., Giakoumi, S., Vardakas, L., Barbieri, R., Stoumboudi, M.T. & Zogaris, S. 2007. The freshwater ichthyofauna of Greece – an update based on a hydrographic basin survey. Mediterranean Marine Science 8, 91-166.

EEA. 2012. Biogeographic regions in Europe. European Environment Agency (www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-1)

Elvira, B. 2001. Identification of non-native freshwater fishes established in Europe and assessment of their potential threats to the biological diversity. Convention on the Conservation of European Wildlife and Natural Habitats, Strasbourg. (Bern\T-PVS 2001\tpvs06e_2001)

Fobert, E., Zięba, G., Vilizzi, L., Godard, M.J., Fox, M.G., Stakėnas, S. & Copp, G.H. 2013. Predicting non-native fish dispersal under conditions of climate change: case study in England of dispersal and establishment of pumpkinseed *Lepomis gibbosus* in a floodplain pond. *Ecology of Freshwater Fish* **22**, 106–116.

Fritschie, K.J. & Olden, J.D. 2016. Non-native introductions influence fish body size distributions within a dryland river. Ecosphere 7(12), e01615. 10.1002/ecs2.1615

García-Berthou, E., Alcaraz, C., Pou-Rovira, Q., Zamora, L., Coenders, G., Feo, C. 2005. Introduction pathways and establishment rates of invasive aquatic species in Europe. Can. J. Fish. Aquat. Sci. 62, 453–463.

García-Llorente, M., Martín-López, B., González, J.A., Alcorlo, P. & Montes, C. 2008. Social perceptions of the impacts and benefits of invasive alien species: Implications for management. Biological Conservation *141*, 2969–2983.

Glamuzina, B., Tutman, P., Nikolić, V., Vidović, Z., Pavličević J., Vilizzi, L. & Copp, G.H. & Simonović, P. 2017. Comparison of taxon-specific and taxon-generic risk screening tools for identifying potentially invasive non-native fishes in the River Neretva catchment (Bosnia & Herzegovina and Croatia). River Research and Applications 33, 670–679.

Gozlan, R.E., Andreou, D., Asaeda, T., Beyer, K., Bouhadad, R., Burnard, D., Caiola, N., Cakic, P., Djikanovic, V., Esmaeili, R., Falka, I., Golicher, D., Harka, A., Jeney, G., Kovácˇ, V., Musil, J., Povz, M., Nocita, A., Poulet, N., Virbickas, T., Wolter, C., Tarkan, A., Tricarico, E., Trichkova, T., Verreycken, H., Witkowski, A., Zhang, C., Zweimueller, I., Britton, J.R., 2010b. Pancontinental invasion of *Pseudorasbora parva*: towards a better understanding of freshwater fish invasions. Fish and Fisheries 11, 315–340.

Grapputo, A., Bisazza, A. & Pilastro, A. 2006. Invasion success despite reduction of genetic diversity in the European populations of eastern mosquitofish (Gambusia holbrooki). Italian Journal of Zoology 73, 67–73.

Green, A.J. & Elmberg, J. 2014. Ecosystem services provided by waterbirds. Biological Reviews 89, 105–122.

Haynes JL & Cashner RC. 1995. Life-history and population- dynamics of the western mosquitofish: a comparison of natural and introduced populations. Journal of Fish Biology 46, 1026–1041.

Holmlund, C.M. & Hammer, M. 1999. Ecosystem services generated by fish populations. Ecological Economics 29, 253–268.

Haas, R. & Pal, R. 1984. Mosquito larvivorous fishes. Bull. Entomol. Soc. Amer. 30, 17–25.

Haas, R.C., Thomas, M.V. & Towns, G.L. 20103. An assessment of the potential use of *Gambusia* for mosquito control in Michigan. Fisheries Division, Technical Report No. 2003-2. Michigan State Department of Natural Resources

Hurlbert, S.H., Zedler, J. & Fairbanks, P. 1972. Ecosystem alteration by mosquito fish (*Gambusia*) predation. Science 175, 639–641.

Johnson, C.R. 1978. The effects of sublethal concentrations of five organophosphorus insecticides on temperature tolerance, reflexes, and orientation in *Gambusia affinis affinis* (Pisces: Poeciliidae). Zoological Journal of the Linnean Society 64, 63–70.

Johnson, L. 2008. Pacific Northwest Aquatic Invasive Species Profile: Western mosquitofish (*Gambusia affinis*) (http://depts.washington.edu/oldenlab/wordpress/wp-content/uploads/2013/03/Gambusia-affinis_Johnson.pdf)

Kalogianni E., Giakoumi S., Andriopoulou A. & Chatzinikolaou Y. 2014. Prey utilisation and trophic overlap between the non native mosquitofish and a native fish in two Mediterranean rivers. Mediterranean Marine Science 15, 287–301.

Kalogianni, E., Giakoumi, S & Chatzinikolaou, Y. 2012. Impacts of the invasive mosquito fish *Gambusia holbrooki* on the Greek native Valencia letourneuxi. XIV European Congress of Ichthyology, Liège, Belgium, 3-8 July 2012. Book of Abstracts, p. 111.

Landeka,. N., Podnar, M. & Jelić, D. 2015. New data on the taxonomic status and distribution of *Gambusia* sp. in Croatia and Bosnia and Herzegovina. Periodicum biologorum 117: 415–424.

Lloyd, L.N., Arthington, A.H. & Milton, D.A., 1986. The mosquitofish — a valuable mosquito-control agent or a pest. *The Ecology of Exotic Animals and Plants some Australian Case Histories*. Wiley, Brisbane, pp. 6–25.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group (ISSG), World Conservation Union (IUCN), 12 pp.

Krumholz, L.A. 1944. Northward acclimatization of the western mosquitofish, *Gambusia affinis*. Copeia 1944:82-85.

Lynch, K.A. 2008. Ecology, Population Genetics and Risk Assessment of the Exotic Mosquitofish, *Gambusia holbrooki*; in Tasmania. PhD thesis, University of Tasmania, 145 pp.

Mata, P.R. & Núñez de Arenas, J.H. 2000. Planes de recuperación en peces en la comunidad valenciana: el fartet y el samaruc. [Recovery plans for fish in the Valencian community: Spanish toothcarp and Valencia toothcarp]. Publicaciones de Biología de la Universidad de Navarra, Serie Zoológica 26, 17–30. (www.unav.es/unzyec/sz/sz26_2.htm)

Macdonald, J. & Tonkin, Z. 2008. A review of the impact of eastern *Gambusia* on native fishes of the Murray-Darling Basin. Murray–Darling Basin Authority Publication 38/09. (www.mdba.gov.au/sites/default/files/pubs/Review-of-gambusia-report.pdf)

Maceda-Veiga, A., Escribano-Alacid, J., de Sostoa, A. & García-Berthou, E. 2013. The aquarium trade as a potential source of fish introductions in southwestern Europe. Biological invasions 15, 2707–2716.

Murphy, C.A., Grenouillet, G. & García-Berthou, E. 2015. Natural abiotic factors more than anthropogenic perturbation shape the invasion of Eastern Mosquitofish (*Gambusia holbrooki*). Freshwater Science 34, 965–974.

Nico, L., P. Fuller, G. Jacobs, M. Cannister, J. Larson, A. Fusaro, T.H. Makled & M. Neilson. 2017. *Gambusia affinis*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL, USA: https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=846 (Revision Date: 1/25/2016)

Oliva-Paterna, F.J., Doadrio, I. & Fernández-Delgado, C. 2006. Threatened fishes of the world: *Aphanius baeticus* (Doadrio, Carmona & Fernández Delgado, 2002)(Cyprinodontidae). Environmental Biology of Fishes 75, 415–417.

Orueta, J.F. 2003. Manual práctico para el manejo de vertebrados invasores en islas de España y Portugal. Gobierno de las Islas Baleares y Gobierno de Canarias, 254. (http://www.researchgate.net/profile/Jorge_Orueta/publication/236629147_Manual_de_manejo_de_vertebrados_invasores_en_islas/links/0c96052653d278acb6000000.pdf

P.H., Soupir, M.L., Biswas, S. and Singh, V.P. 2014. Contamination of water resources by pathogenic bacteria. AMB Express, 4(1), 51. (www.amb-express.com/content/4/1/51)

Peel, M.C., Finlayson, B.L. & McMahon, T.A. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences Discussions* 4, 439–473.

Perdikaris, C., Koutsikos, N., Vardakas, L., Kommatas, D., Simonović, P., Paschos, I., Detsis, V., Vilizzi, L. & Copp, G.H. 2016. Risk screening of non-native, translocated and traded aquarium freshwater fishes in Greece using the Fish Invasiveness Screening Kit. Fisheries Management and Ecology 23, 32–43.

Pimentel, D., Lach, L., Zuniga, R. and Morrison, D., 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience 50, 53–65.

Pino-del Carpio, A., Miranda, R. & Puig, J. 2010. Non-native freshwater fish management in biosphere reserves. *Management of Biological Invasions* 1, 13–33.

Piria, M., Povž, M., Vilizzi, L., Zanella, D., Simonović, P. & Copp, G.H. 2016. Risk screening of non-native freshwater fishes in Croatia and Slovenia using the Fish Invasiveness Screening Kit. Fisheries Management and Ecology 23, 21–31.

Pyke, G.H. 2005. A review of the biology of Gambusia affinis and G. holbrooki. Reviews in Fish Biology and Fisheries 15, 339-365.

Pyke, G.H. 2008. Plague minnow or mosquito fish? A review of the biology and impacts of introduced Gambusia species. Ann Rev Ecol Syst 39, 171–191.

Reddy, S.R. & Pandian, T.J. 1972. Heavy mortality of *Gambusia affinis* reared on diet restricted to mosquito larvae. Mosquito News 32, 108–110.

Reshetnikov, A.N. 2004. The fish *Perccottus glenii*: history of introduction to western regions of Eurasia. Hydrobiologia 522: 349–350

Rincón P.A., Correas A.M., Morcillo F., Risueño P. & Lobón-Cerviá J. 2002. Interaction between the introduced Eastern mosquitofish and two autochthonous Spanish toothcarps. Journal of Fish Biology 61, 1560–1585.

Rowe, D.K., Moore, A., Giorgetti, A., Maclean, C., Grace, P., Wadhwa, S., Cooke, J. 2008. Review of the impacts of gambusia, redfin perch, tench, roach, yellowfin goby and streaked goby in Australia. Prepared for the Australian Government Department of the Environment, Water, Heritage and the Arts. Common- wealth of Australia, 245 pp. (www.environment.gov.au/biodiversity/publications/index.html)

Rupp, H.R. 1996. Adverse assessments of *Gambusia affinis*: an alternate view for mosquito control practitioners. Journal of the American Mosquito Control Association, 12(2 Pt 1), 155–159.

Sanz, N., Araguas, R.M., Vidal, O., Diez-del-Molino, D., Fernández-Cebrián, R. and García-Marín, J.L. 2013. Genetic characterization of the invasive mosquitofish (*Gambusia* spp.) introduced to Europe: population structure and colonization routes. Biological Invasions 15, 2333–2346.

Schmidt, B., 2013. Transportieren Enten Fische in natürlicherweise fischfreie Amphibienlaichgebiete. Zeitschrift für Feldherpetologie, 20, 137–144.

Silva, J.P., Toland, J., Nottingham, S., Jones, W., Eldridge, J., Hudson, T., Heppner, K., McGlynn, D. & Thévignot, C. 2015. Life and Freshwater Fish. European Commission, 65 pp. (doi:10.2779/245852) (ec.europa.eu/environment/life/publications/lifepublications/lifefocus/.../fish.pdf)

Simonović, P., Tošić, A., Vassilev, M., Apostolou, A., Mrdak, D., Ristovska, M., Kostov, V., Nikolić, V., Škraba, D., Vilizzi, L. & Copp, G.H. 2013. Risk assessment of non-native fishes in the Balkans Region using FISK, the invasiveness screening tool for non-native freshwater fishes. Mediterranean Marine Science 14, 369–376.

Specziár, A. 2004. Life history pattern and feeding ecology of the introduced eastern mosquitofish, *Gambusia holbrooki*, in a thermal spa under temperate climate, of Lake Heviz, Hungary. Hydrobiologia 522, 249–260.

Srean, P. 2015. Understanding the ecological success of two worldwide fish invaders (*Gambusia holbrooki* and *Gambusia affinis*). PhD dissertation, Univ. of Girona, Spain (http://hdl.handle.net/10803/295975)

Sutherst, R.W. 2004. Global change and human vulnerability to vector-borne diseases. Clinical microbiology reviews 17(1), 136–173.

Tarkan, A.S., Ekmekçi, G., Vilizzi, L. & Copp, G.H. 2014. Risk screening of non-native freshwater fishes at the frontier between Asia and Europe: first application in Turkey of the Fish Invasiveness Screening Kit (FISK). Journal of Applied Ichthyology 30, 392–398.

Towns, G.L. 1977. Overwintering and lower lethal temperature and oxygen levels of the mosquitofish, *Gambusia affinis* (Baird and Girard), and its effectiveness in controlling mosquito larvae in woodland vernal ponds near Mt. Pleasant, Michigan. MSc. Thesis, Central Michigan University, Mount Pleasant.

Turbelin, A.J., Malamud, B.D. & Francis, R.A. 2017. Mapping the global state of invasive alien species: patterns of invasion and policy responses. Global Ecology and Biogeography 26, 78–92.

Vidal, O., García-Berthou, E., Tedesco, P.A. & García-Marín, J.L. 2010. Origin and genetic diversity of mosquitofish (*Gambusia holbrooki*) introduced to Europe. Biological Invasions 12(4), 841–851.

Vooren, C. 1971. Ecological aspects of the introduction of fish species into natural habitats in Europe, with special reference to the Netherlands. J. Fish Biol. 4, 565–583.

Wach, E. & Chambers, R.M. 2007. Top-down effect of fish predation in Virginia headwater streams. Northeastern Naturalist 14, 461–470.

Wallace, A.R. (1876). *The Geographical Distribution of Animals with a Study of the Relations of Living and Extinct Faunas as Elucidating the Past Changes of the Earth's Surface*. Macmillan & Co., London

Walton, W.E., Henke, J.A. & Why, A.M. 2012. *Gambusia affinis* (Baird and Girard) and *Gambusia holbrooki* Girard (Mosquitofish). pp. 261–273 In: R.A. Francis (ed.) *A Handbook of Global Freshwater Invasive Species*. EarthScan from Routledge, London.

Wheeler, A.C., Merrett, N.R. & Quigley, D.T.G. 2004. Additional records and notes for Wheeler's (1992) List of the common and scientific names of fishes of the British Isles. Journal of Fish Biology 65 (Supplement B), 1–40.

Willis, K. & Ling, N. 2000. Sensitivities of mosquitofish and black mudfish to a piscicide: could rotenone be used to control mosquitofish in New Zealand wetlands?. New Zealand Journal of Zoology 27(2), 85–91.

Wooten, M.C., Scribner, K.T. & Smith, M.H. (1988) Genetic variability and systematics of *Gambusia* in the southeastern United States. Copeia 1988, 283–289.

Zarev, V.Y. 2012. Some life-history traits of *Gambusia holbrooki* (Pisces: Poeciliidae) from Bulgaria. Acta Zoologica Bulgarica 64, 263–272.

Specziár, A. 2004. Life history pattern and feeding ecology of the introduced eastern mosquitofish, *Gambusia holbrooki*, in a thermal spa under temperate climate, of Lake Heviz, Hungary. Hydrobiologia 522, 249–260.

Zięba, G., Copp, G.H., Davies, G.D., Stebbing, P.D., Wesley, K.J. & Britton, J.R. 2010. Recent releases and dispersal of non-native fishes in England and Wales, with emphasis on sunbleak *Leucaspius delineatus*. Aquatic Invasions 5, 155–161.

Zogaris, S. 2014. Ichthyological Study for Oroklini Lake. April 2014. BirdLife Cyprus. A study within the Project LIFE10 NAT/CY/716 "Restoration and Management of Oroklini Lake SPA, Larnaca". Unpublished Final Report, 58 pp. Nicosia, Cyprus.

Zogaris, S. 2017. Conservation study of the Mediterranean Killifish *Aphanius fasciatus* in Akrotiri Marsh (Akrotiri SBA, Cyprus), Final Report.

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[1]	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

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

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

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

ANNEX IV - Species Distribution Model

Data for modelling

Climate data were taken from freshwater-specific versions of the 'Bioclim' variables (Domisch *et al.*, 2015), aggregated to a 0.25 x 0.25 degree grid for use in the model. Based on the biology of the two species (Pyke, 2005; Vondracek, 1988), the following climate variables were used in the modelling:

- Mean upstream temperature of the coldest month (Bio6 °C) reflecting the winter cold stress.
- Mean upstream temperature of the warmest quarter (Bio10 °C) reflecting the summer thermal regime.
- Mean upstream annual precipitation (Bio12 mm) was used as an indicator of the availability of aquatic habitats.
- Upstream precipitation of the driest quarter (Bio17 mm) was used as an indicator of low flows, which might be detrimental for the species.

Climate model projections of climate change scenarios for these freshwater-specific variables are currently unavailable so no climate change projections could be made.

In the models we also included the following habitat variables, all ln+1 transformed for modelling:

- Density of permanent rivers was estimated from the Vector Map (VMAP0; http://gis-lab.info/qa/vmap0-eng.html). River vectors were rasterised at 0.02 x 0.02 degree resolution. Then, the percentage of these grid cells containing rivers within each of the 0.25 x 0.25 degree cells used in the model was calculated.
- % Cover of lakes and wetlands from the Global Lakes and Wetlands Database (Lehner & Döll, 2004) processed similarly to the above.
- Average slope derived from the Hydrosheds database (Lehner *et al.*, 2006) and available with the freshwater-specific climate data (Domisch *et al.*, 2015).

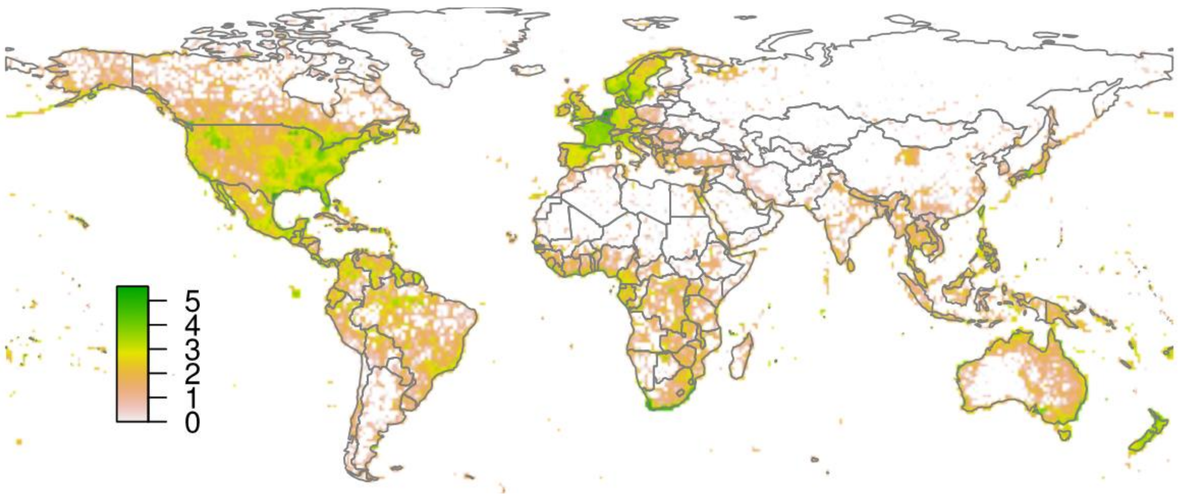
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF), USGS Biodiversity Information Serving Our Nation (BISON), iNaturalist and Berkeley Ecoengine, Vertnet, iDigBio and Atlas of Living Australia. The records were scrutinised to remove unreliable occurrences. This included re-classifying all European records of *G. affinis* as *G. holbrooki* as the species have been historically misidentified in Europe. Records were then gridded at a 0.25 x 0.25 degree resolution for modelling (Figure 1a). In total 2577 grid cells containing records of *G .affinis* and 1718 of *G. holbrooki* were used in the modelling (Figure 1a). Native range polygons for both species were obtained from the IUCN Red List for *G. holbrooki* and adapted from NatureServe for *G. affinis*. Additionally, the recording density of Actinopterygii on GBIF was obtained as a proxy for spatial recording effort bias (Figure 1b).

Figure 1. Occurrence records obtained for *Gambusia affinis* and (b) *G. holbrooki* used in the modelling, showing the native range and (c) a proxy for recording effort – the number of Actinopterygii records held by the Global Biodiversity Information Facility, displayed on a log_{10} scale.

(a) Distribution of *Gambusia affinis* used in modelling

(b) Distribution of *Gambusia holbrooki* used in modelling

(c) Estimated recording effort (log_{10}-scaled)



Species distribution model

For both species, a presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7 (Thuiller *et al.*, 2014, Thuiller *et al.*, 2009). These models contrast the environment at the species' occurrence locations against a random sample of background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to. Therefore the background sampling region included:

- The area accessible by native populations, in which the species is likely to have had sufficient time to disperse to all locations. A 100 km buffer around the native range polygons shown in Figure 1 was assumed to be accessible; AND
- A small 30 km buffer around all non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species (see Figure 2). Absence from these regions is considered to be irrespective of dispersal constraints. Based on extreme values at the occurrence locations, we specified conditions appearing to be too cold, dry or steep for each species. For *G. affinis* these rules were:

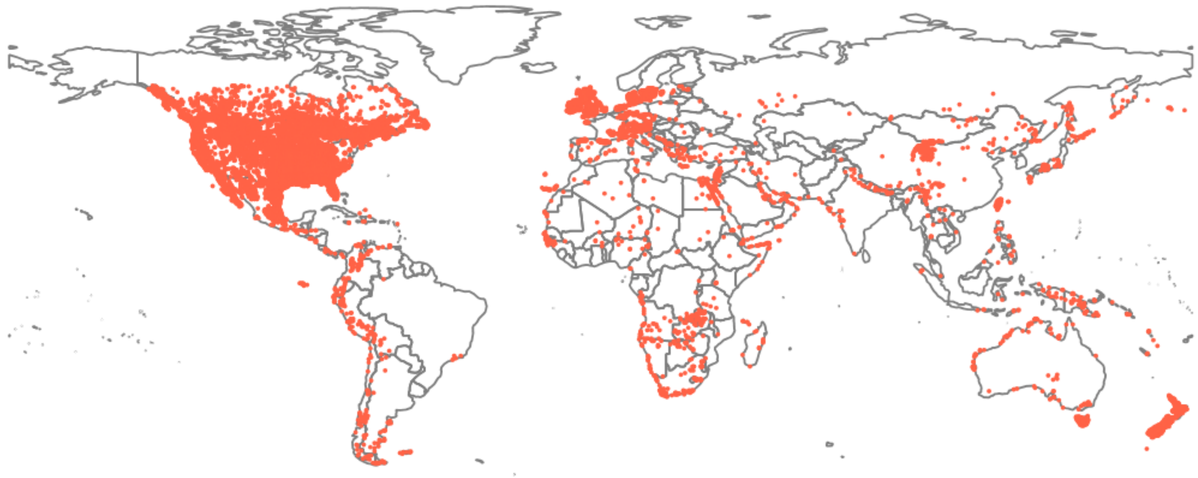
  - Mean upstream temperature of the coldest month (Bio6) < -11 °C.
  - Mean upstream temperature of the warmest quarter (Bio10) < 16 °C.
  - Mean upstream annual precipitation (Bio12) < 30000 mm.
  - Upstream precipitation of the driest quarter (Bio17) < 500 mm.
  - Slope > 8 degrees.
- For *G. holbrooki* the rules were:
  - Mean upstream temperature of the coldest month (Bio6) < -6 °C.
  - Mean upstream temperature of the warmest quarter (Bio10) < 17 °C.
  - Mean upstream annual precipitation (Bio12) < 25000 mm.
  - Upstream precipitation of the driest quarter (Bio17) < 2000 mm.
  - Slope > 10 degrees.

Very few occurrence grid cells fell within the regions deemed unsuitable based on these rules – 1.2% for *G. affinis* and 2.3% for *G. holbrooki*.

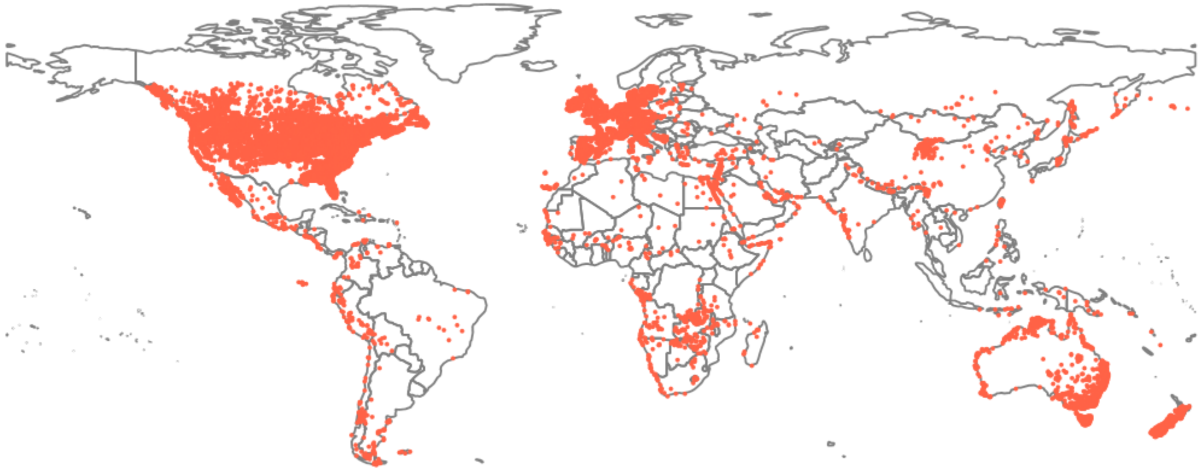
To sample as much of the background environment as possible, without overloading the models with too many pseudo-absences, ten background samples of 5,000 randomly chosen grid cells were obtained (Figure 2). To account for recording effort bias, sampling of background grid cells was weighted in proportion to the reptile recording density (Figure 1c).

Figure 2. Randomly selected background grid cells used in the modelling of both species, mapped as points. Points are sampled from the native range, a small buffer around non-native occurrences and from areas expected to be highly unsuitable for the species, and weighted by a proxy for recording effort (Figure 1c).

(a) *Gambusia affinis*



(b) *Gambusia holbrooki*



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, ten statistical algorithms were fitted with the default BIOMOD2 settings (except where specified below) and rescaled using logistic regression:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per effect.
- Classification tree algorithm (CTA)
- Artificial neural network (ANN)
- Flexible discriminant analysis (FDA)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent
- Maximum entropy multinomial logistic regression (MEMLR)

Since the background sample was much larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure. Model predictive performance was assessed by calculating the Area Under the Receiver-Operator Curve (AUC) for model predictions on the evaluation data, which were reserved from model fitting. AUC can be interpreted as the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence.

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability.

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

Limiting factor maps were produced following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell. Partial response plots were also produced by predicting suitability across the range of each predictor, with other variables held at near-optimal values.

Results

The ensemble model suggested that at the scale of the modelling the suitability for both species was most strongly determined by the proxies for water temperature and flow, with little effect of freshwater habitat or slope (Table 1). The influence of the unsuitability rules on the modelling was clearly evident in the modelled response functions (Figures 3 and 4).

Global projection of the model in current climatic conditions (Figures 5 and 6) indicates that both species may be capable of substantial further range expansion beyond their native ranges in North America. Of the two species, *G. affinis* has a larger modelled potential distribution in North America (Figures 5 and 6), consistent with its more extensive actual distribution (Figure 1). Beyond North America, many other parts of the world have climates that are apparently suitable for the species, especially throughout warm temperate regions (Figures 5 and 6), including much of southern Europe (Figure 7). Suitability for the species in northern Europe was predicted to be limited by summer temperatures that are cooler than most places in which the species are currently found (Figure 8). In north eastern Europe suitability was predicted to be limited by cold winters, especially for *G. holbrooki* (Figure 8).

The Biogeographical Region of Europe (Bundesamt fur Naturschutz (BfN), 2003) predicted to be most suitable to both species was the Mediterranean (Figure 9). Other regions with high suitability for the species included the Anatolian region for *G. affinis* and the Black Sea region for *G. holbrooki* (Figure 9).

Caveats to the modelling

Modelling the potential global distributions of range-expanding species is always difficult and uncertain. Both the species considered here exhibits invasive (adventive) behaviour in their native continent, implying that there are strong natural dispersal constraints on their native North American distributions. Even though the modelling techniques used here are designed to account for dispersal constraints, these may have impeded our ability to characterise climatic limits on establishment of the species if the species can tolerate wider climatic ranges than are experienced in their dispersal-limited ranges. Both species are considered highly tolerant, adaptable and variable at both individual and population levels (Pyke, 2005) and so may be capable of establishing more widely than is observed in their current distribution and projected here.

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

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

Species	Algorithm	AUC	In the ensemble	Minimum temperature of coldest month	Mean temperature of warmest quarter	Annual precipitation	Precipitation of driest quarter	River density	Wetland cover	Slope
G. affinis	GLM	0.8040	yes	17%	45%	23%	12%	0%	0%	2%
	GBM	0.8183	yes	25%	64%	2%	3%	0%	0%	5%
	GAM	0.8092	yes	22%	36%	25%	13%	0%	0%	4%
	CTA	0.7943	yes	23%	57%	3%	7%	1%	1%	7%
	ANN	0.8270	yes	18%	36%	22%	18%	0%	1%	4%
	FDA	0.8143	yes	10%	51%	24%	13%	0%	0%	1%
	MARS	0.8117	yes	18%	44%	23%	11%	0%	0%	4%
	RF	0.6780	no	19%	50%	7%	6%	4%	4%	9%
	Maxent	0.8118	yes	31%	49%	8%	5%	0%	1%	6%
	MEMLR	0.7120	no	7%	15%	28%	25%	0%	5%	20%
	Ensemble	**0.8212**		**21%**	**48%**	**16%**	**10%**	**0%**	**1%**	**4%**
G. holbrooki	GLM	0.8856	yes	47%	44%	0%	8%	0%	0%	0%
	GBM	0.8906	yes	46%	42%	0%	11%	0%	0%	1%
	GAM	0.8908	yes	44%	40%	5%	9%	0%	0%	0%
	CTA	0.8612	no	43%	42%	0%	14%	0%	0%	1%
	ANN	0.8935	yes	37%	35%	10%	15%	0%	1%	2%
	FDA	0.8862	yes	45%	41%	0%	13%	1%	0%	0%
	MARS	0.8884	yes	46%	43%	0%	11%	0%	0%	0%
	RF	0.8181	no	41%	37%	2%	10%	3%	3%	5%
	Maxent	0.8868	yes	42%	39%	3%	12%	1%	1%	2%
	MEMLR	0.8305	no	23%	13%	31%	31%	2%	0%	0%
	Ensemble	**0.8937**		**44%**	**41%**	**3%**	**11%**	**0%**	**0%**	**1%**

Figure 3. Partial response plots from the fitted models for *G. affinis*, ordered from most to least important. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

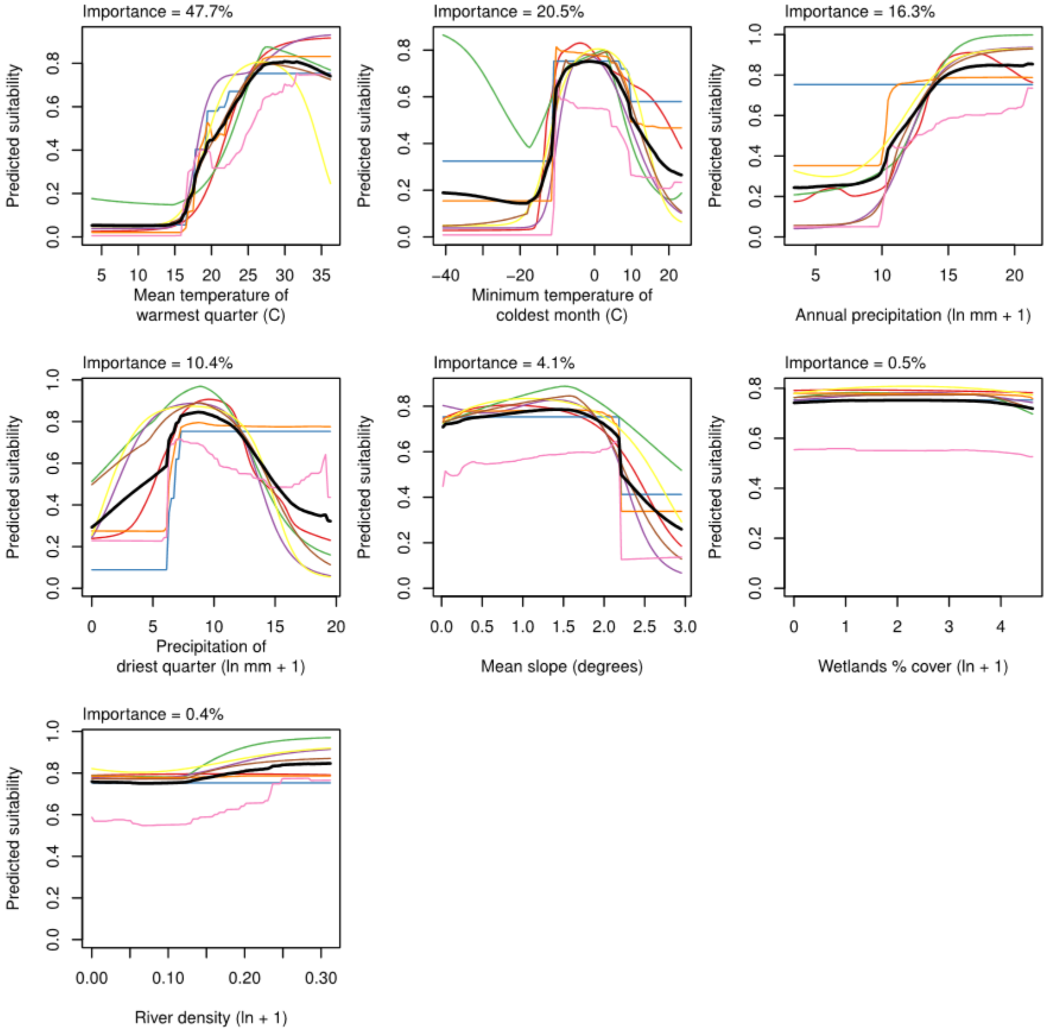


Figure 4. Partial response plots from the fitted models for *G. holbrooki*, ordered from most to least important. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

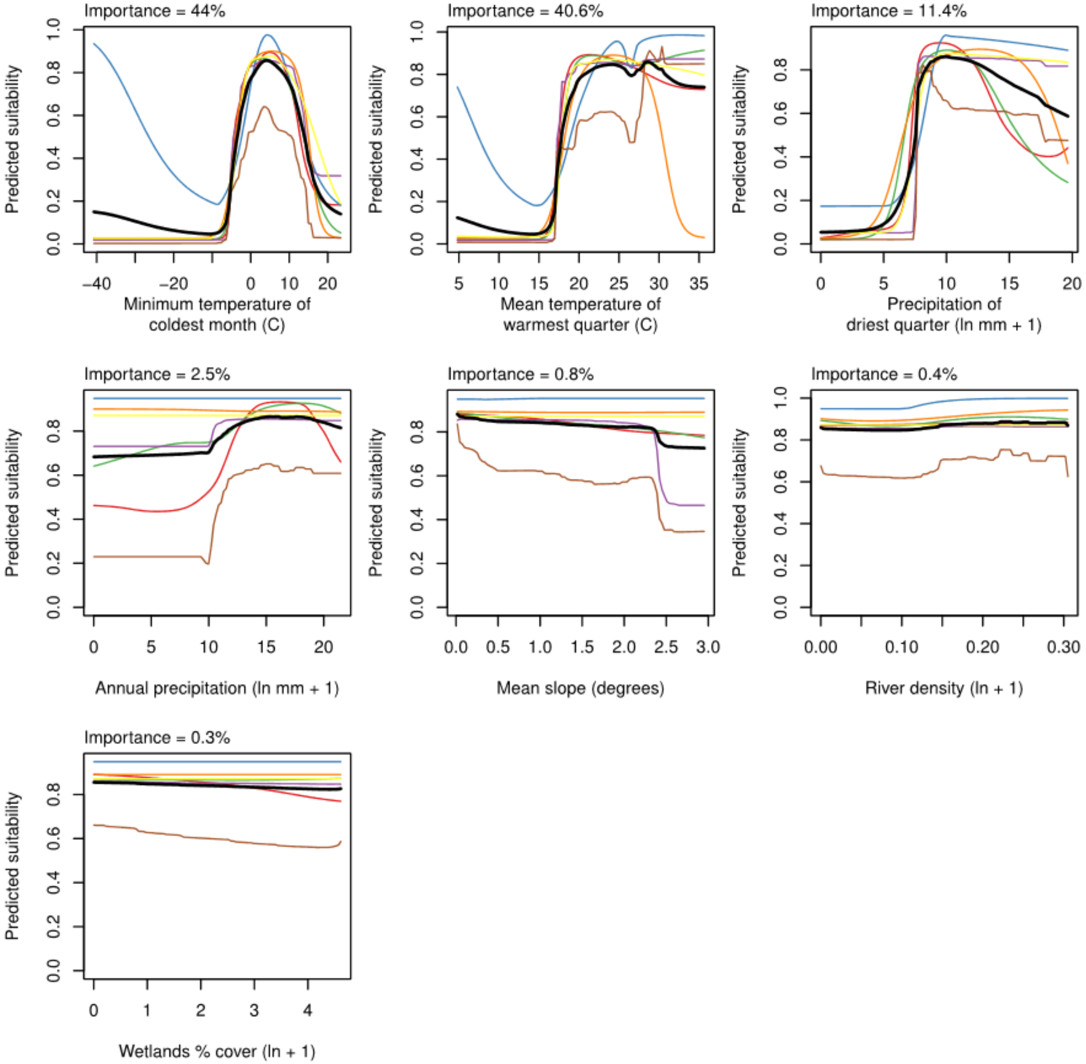


Figure 5. (a) Projected global suitability for *G. affinis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Red shading indicates suitability. White areas have climatic conditions outside the range of the training data so were excluded from the projection. (b) Uncertainty in the suitability projections, expressed as the standard deviation of projections from different algorithms in the ensemble model.

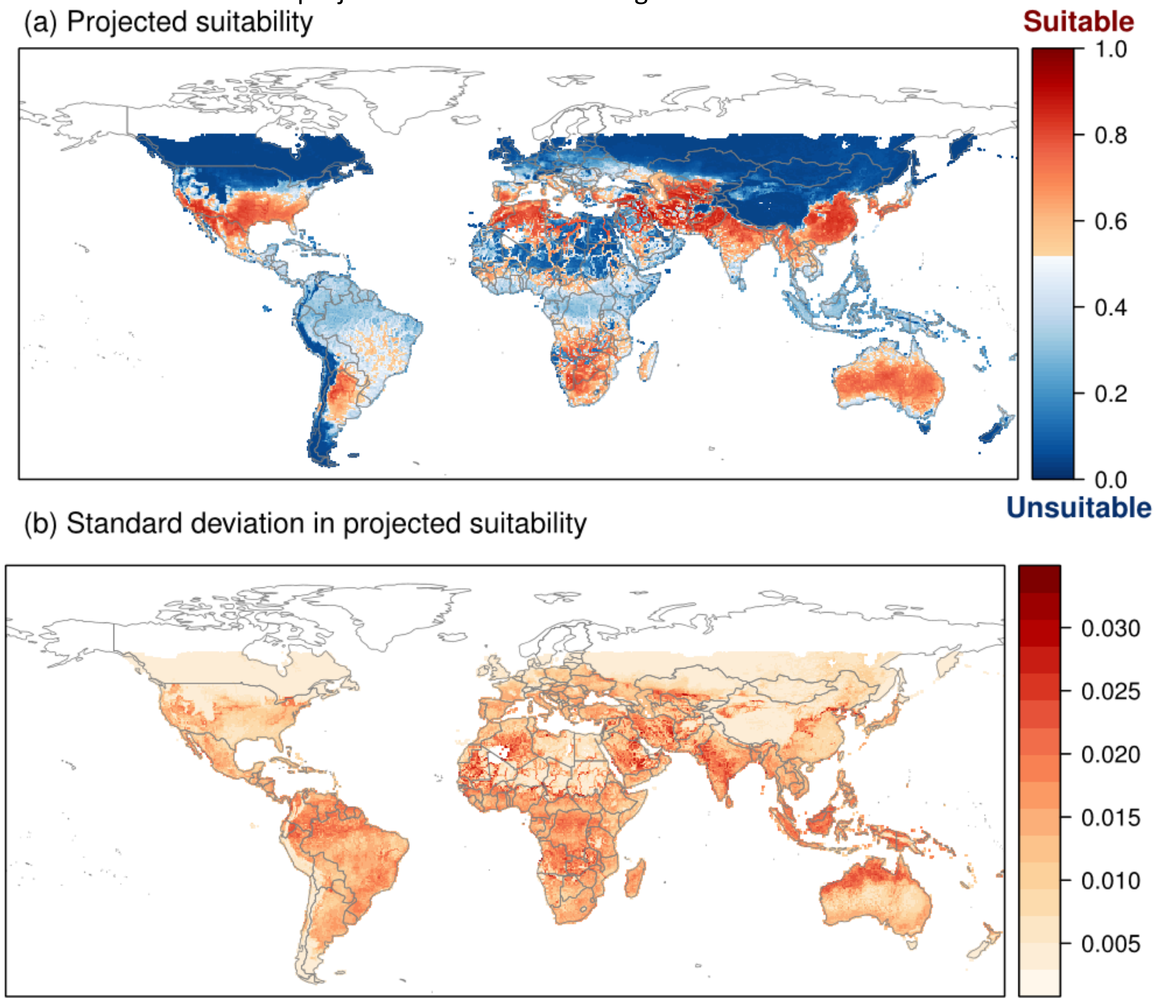


Figure 6. (a) Projected global suitability for *G. holbrooki* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Red shading indicates suitability. White areas have climatic conditions outside the range of the training data so were excluded from the projection. (b) Uncertainty in the suitability projections, expressed as the standard deviation of projections from different algorithms in the ensemble model.

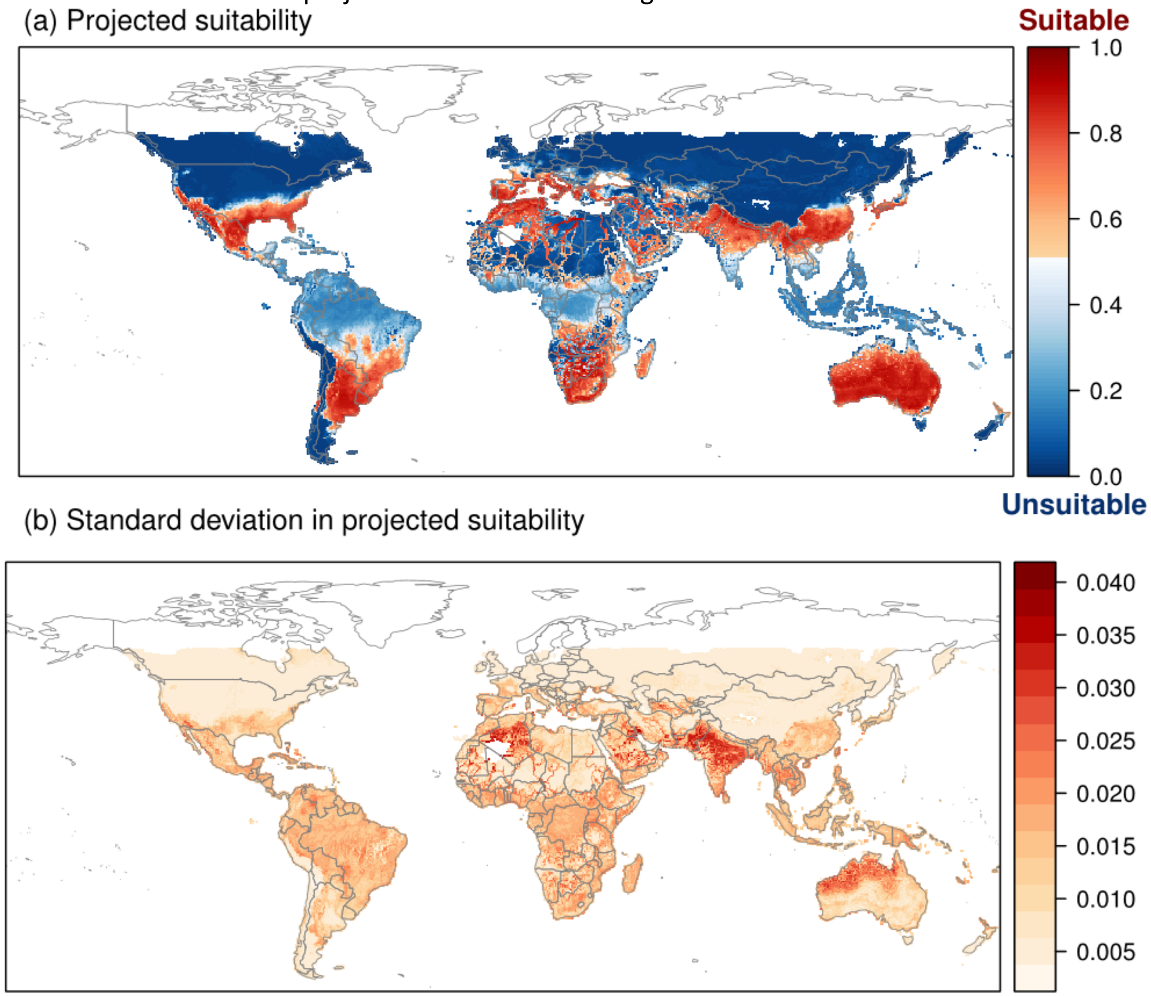
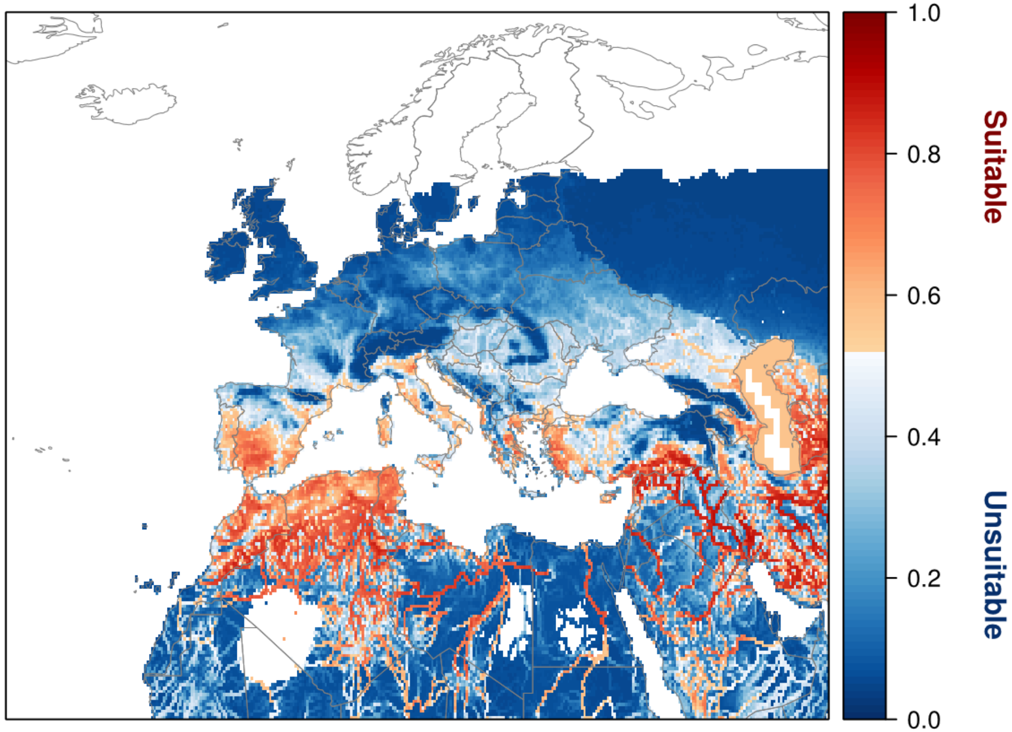


Figure 7. Projected current suitability for (a) *G. affinis* and (b) *G. holbrooki* establishment in Europe and the Mediterranean region. The white areas have climatic conditions outside the range of the training data so were excluded from the projection.

(a) *Gambusia affinis*



(b) *Gambusia holbrooki*

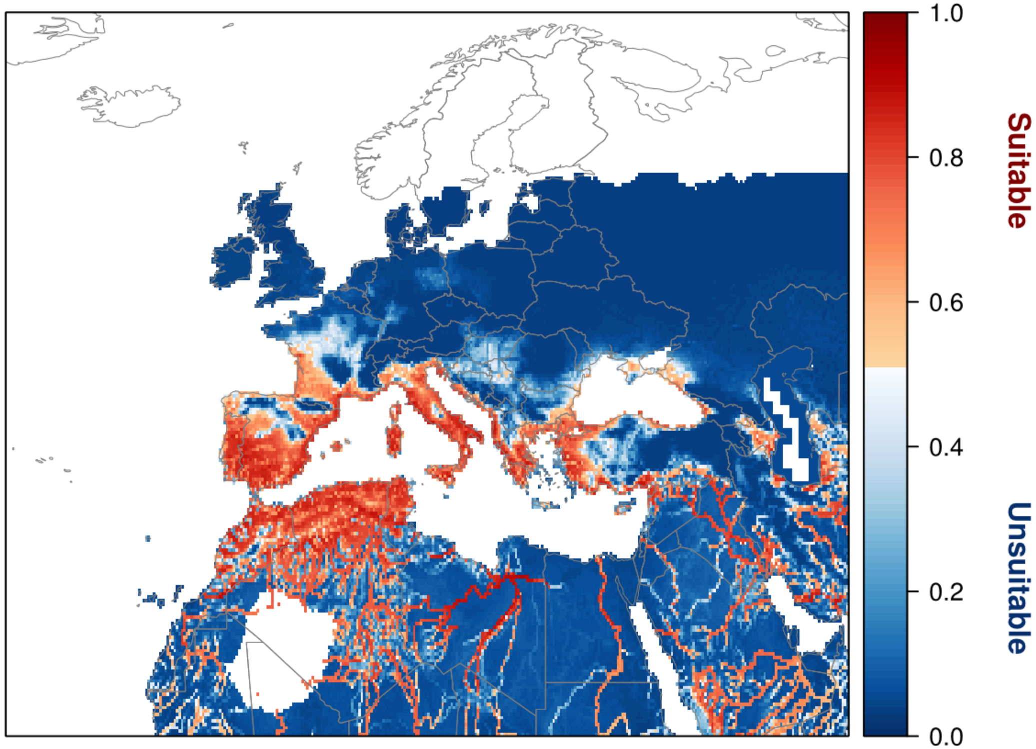
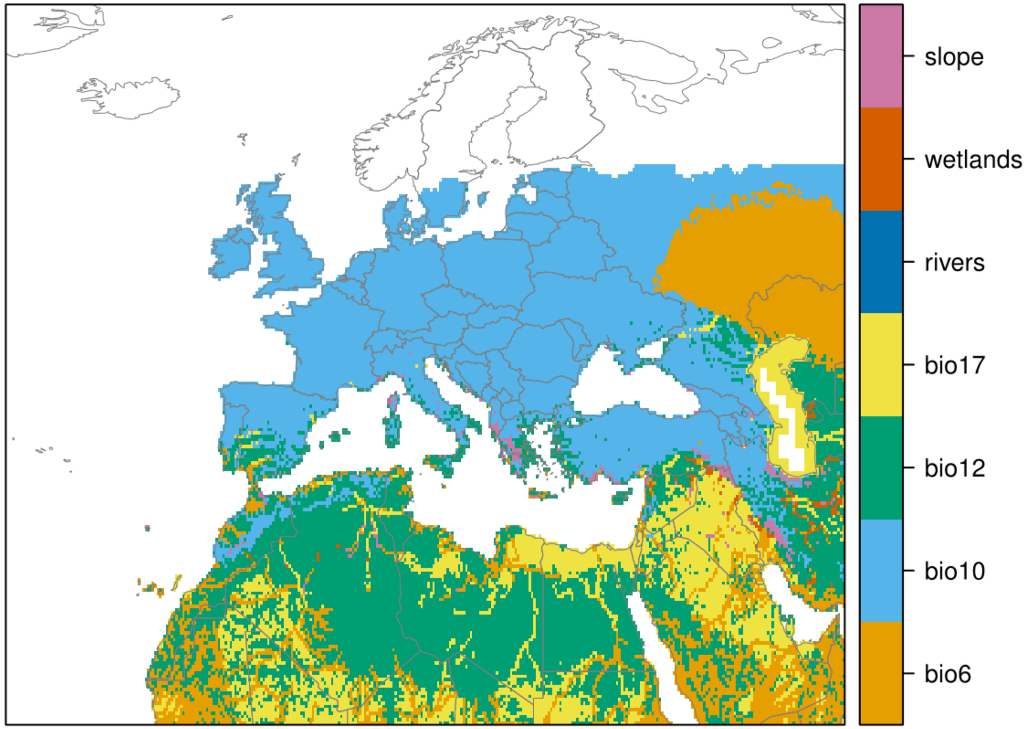


Figure 8. Limiting factor map for (a) *G. affinis* and (b) *G. holbrooki* establishment in Europe and the Mediterranean region in the current climate. Shading shows the predictor variable most strongly limiting projected suitability.

(a) *Gambusia affinis*



(b) *Gambusia holbrooki*

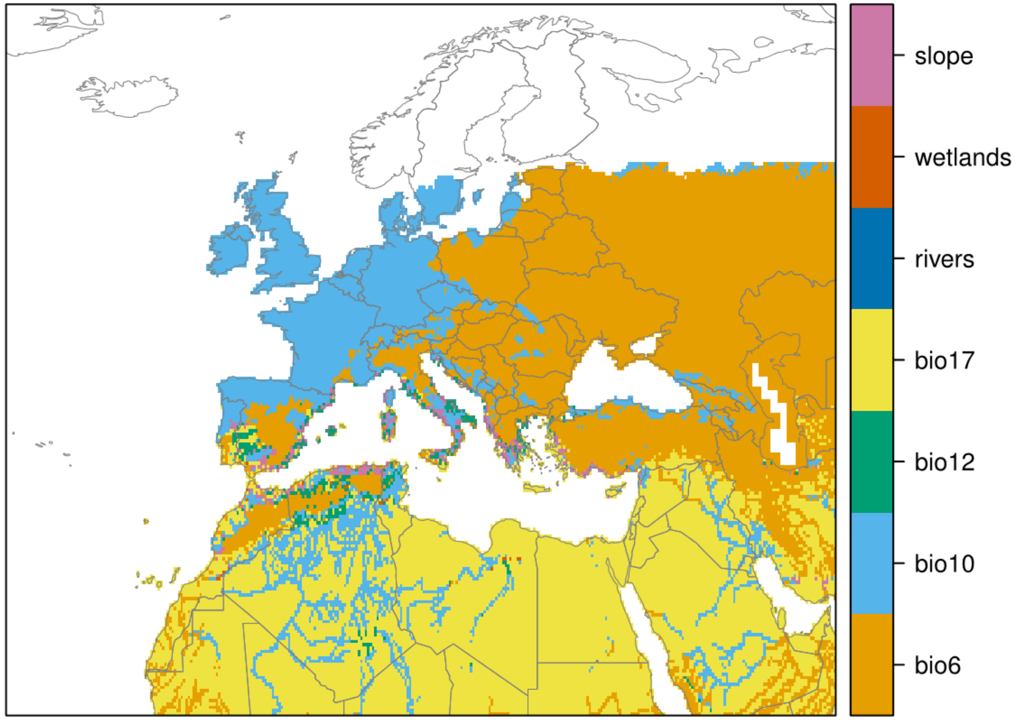
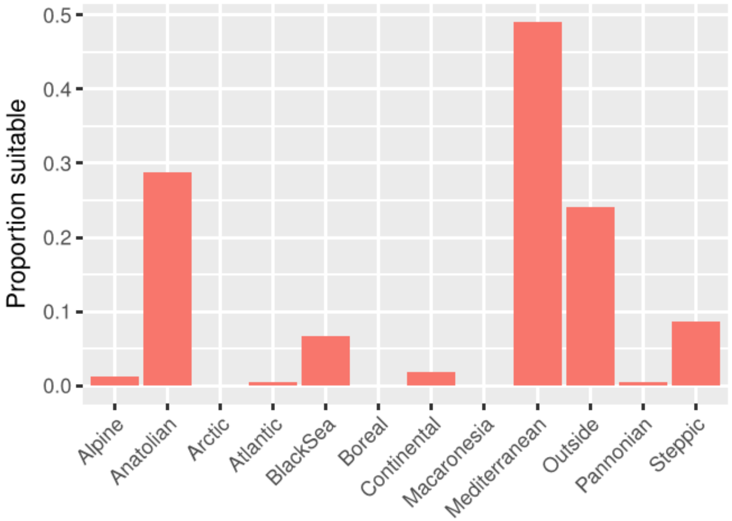
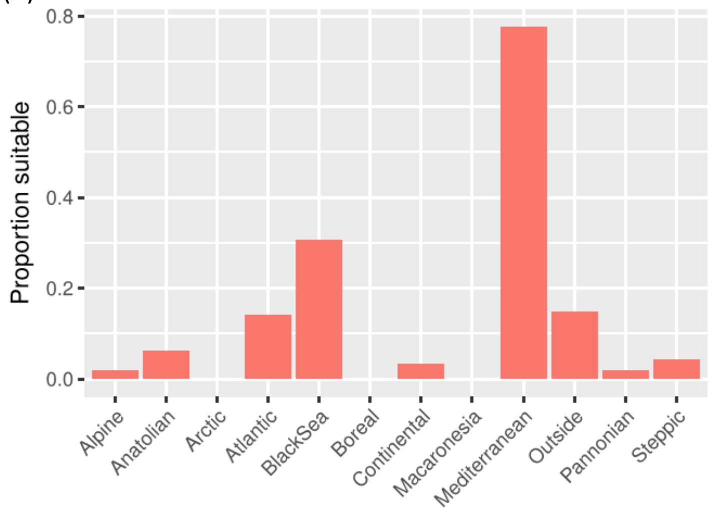


Figure 9. (a-b) Variation in projected suitability for both species among (c) Biogeographical regions of Europe (Bundesamt fur Naturschutz (BfN), 2003). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate.

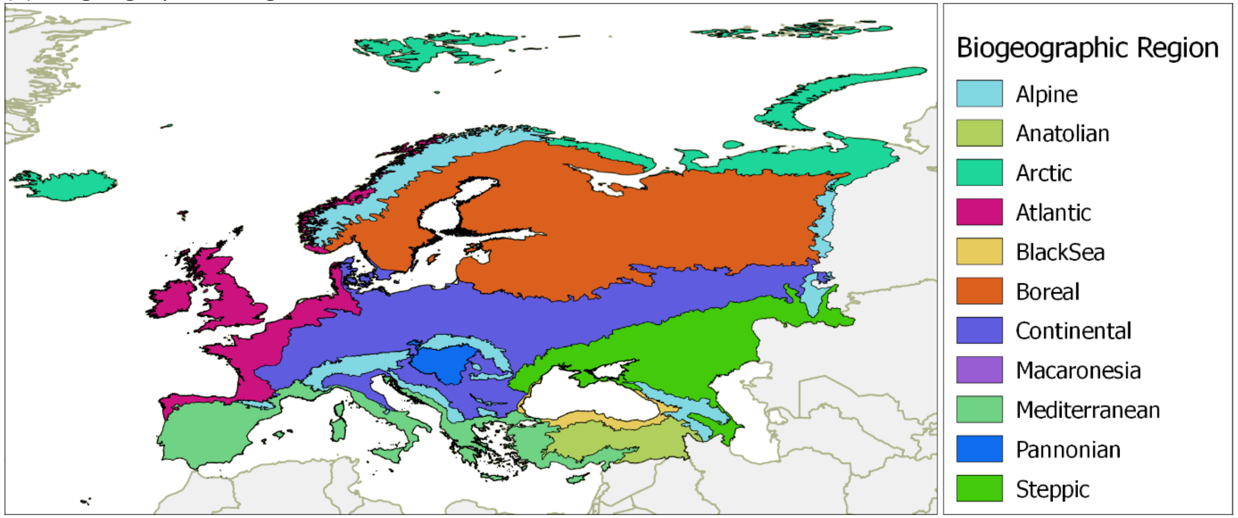
(a) *Gambusia affinis*



(b) *Gambusia holbrooki*



(c) Biogeographical regions



References

S. Domisch, G. Amatulli & W. Jetz (2015) Near-global freshwater-specific environmental variables for biodiversity analyses in 1 km resolution. **2**, 150073.
B. Lehner & P. Döll (2004) Development and validation of a global database of lakes, reservoirs and wetlands. *Journal of Hydrology* **296**, 1-22.
B. Lehner, K. Verdin & A. Jarvis (2006) HydroSHEDS technical documentation, version 1.0. *World Wildlife Fund US, Washington, DC*, 1-27.
W. Thuiller, D. Georges & R. Engler (2014) biomod2: Ensemble platform for species distribution modeling. R package version 3.3-7 *Available at: https://cran.r-project.org/web/packages/biomod2/index.html*.
W. Thuiller, B. Lafourcade, R. Engler & M. B. Araújo (2009) BIOMOD–a platform for ensemble forecasting of species distributions. *Ecography* **32**, 369-373.
G. H. Pyke (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries* **15**, 339-365.

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

Species (common name)	**western mosquitofish**
Species (scientific name)	***Gambusia affinis***
Date Completed	**15 September 2017**
Annex authors	**H. Verreycken and G.H. Copp**
Version	**V1**

	Description of measures[1]	**Assessment of implementation cost and cost-effectiveness (per measure)[2]**	**Level of confidence[3]**
Methods to achieve prevention	**Managing pathways**: *Gambusia affinis* have been introduced to new areas through a variety of pathways, including the pet/aquarium trade and deliberate introductions for biological control. The adoption and enforcement of appropriate legislation and codes of best practice to reduce the risks posed by these pathways should reduce the probability of further introductions	Costs are roughly estimated to be medium for the EU (< €50k?).	Medium
Methods to achieve	**Effective surveillance and reporting**: *Gambusia* is a readily	The following methods may be suitable for surveillance and monitoring in the EU. Electrofishing and fyke-netting are	Medium

eradication	identifiable genus although the species *G. affinis* and *G. holbrooki*, and their hybrids, may be difficult to identify. Effective eradication is most likely to be achieved when new invasions are quickly reported. Reports of new or previously-undiscovered populations in an area is moderately likely to attract public attention, depending upon the isolation of the infested water body and the familiarity of the person(s) with species' identity and its non-native status. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the species can become established. Post-eradication detection can also be undertaken to determine whether or not an eradication action has been successful.	commonly used to monitor fish populations in rivers, canals and lakes. However, seine nets, traps and dip nets are more efficient in catching *G. affinis* and should therefore be used in addition. Also citizen-science species occurrence datasets are increasingly recognized as a valid tool for monitoring the occurrence and spread of invasive species across large spatial and temporal scales (Roy *et al.*, 2015). Post-eradication detection methods should normally combine both conventional and molecular techniques such as environmental DNA (e.g. Davison *et al*. 2017). Cost are low to medium (€5k to €50k). Successful eradications of fish in larger water bodies are very rare.	
	Depletion and/or drain down of small running waters: *G. affinis* are most commonly associated with stillwater environments, but they do occur in water courses. In most EU countries, the use of 'rotenone' piscicide (see here below) is not permitted, so the only options for stillwater environments are depletion sampling or diversion of the water course to permit drainage of the infested reach of the water course.	The following methods may be suitable for depletion sampling and removal of fishes in the EU: electrofishing, seine nets, minnow traps, and fyke nets. All of these except electrofishing are more commonly used in still waters but can be used in water courses. The likelihood of successful eradication, however, is low except in very small and unusual circumstances. For example, the likelihood of success would probably be higher in small, low discharge, slow-flowing water courses with little or no instream encumberments, decreasing as stream size, stream discharge rate, water velocities and the amount of encumberments increase. These two methods are usually used together, first depleting the numbers of the target organism so as to avoid escapees that could result in wider dispersal during the drain down process. However, in cases of a low density	Medium

		invader (i.e. before the species has had a change to overrun the water course, drain-down can be undertaken at a similar risk level (of escapees) as a highly-populated water course that has been depleted down to low density. Also, the depletion process is not solely for reducing the abundance of the target organism, but also potentially to recover native (or other desirable) species prior to the drain down, which would impose a high stress level resulting in the mortality of non-target fishes prior to the complete dewatering of the water course. Cost are likely to be medium to high (>€50k/ha)	
	Use of piscicide: a piscicide can be used to kill newly-detected populations in smaller areas such as ponds, drainable larger water bodies (e.g. reservoirs), or small water courses.	The piscicide 'rotenone' and the draining down and liming of the water body have been applied successfully for eradication of topmouth gudgeon (*Pseudorasbora parva*) in relatively small water bodies in the UK (Britton et al., 2010). However, the use of rotenone would not be acceptable in several EU-countries and derogations of existing legislation to get permission to use rotenone would be difficult to obtain. Thus it would be only manageable in countries where the use of piscicides is allowed. The financial implication of the use of rotenone is not particularly high (e.g. cost of rotenone only GBP20·L^{-1}; in 2017 ≈€20·L^{-1}; the quantity of chemicals depends on the character of the water body and the species to be eradicated), and can be even less if non-rotenone options are both feasible and effective (Britton et al., 2010). However it requires intensive manpower input during key stages (Britton and Brazier, 2006). Some species require a high concentration of rotenone, some simply a longer duration of exposure (e.g. *Pseudorasbora parva* requires 2× the exposure of most other cyprinid fishes to be killed by rotenone; see Allen *et al*. 2006). Rotenone kills all fish species and is also harmful to amphibians and aquatic invertebrates, so collateral damage is high. It is very likely that the general public and/or stakeholders may show	High

		resistance to the approach (on environmental / animal welfare grounds), though this may be possible to overcome through awareness and education of the general public regarding the risks to biodiversity, the environment and ecosystem services posed by invasive non-native species (Bremner and Park, 2007). Although already applied in some EU (e.g. UK) and non-EU countries (e.g. Norway), many countries would not allow the use of piscicides in open waters (note that use of rotenone in the UK is limited in most cases to still waters). Eradication may only be feasible in ponds (Britton and Brazier, 2006, Britton et al., 2010) and small streams (Weyl et al., 2013). *G. affinis* matures at four to six weeks, which means that three generations can be produced in one year, so a close follow-up is necessary, e.g. potentially using environmental DNA (eDNA) methods to complement conventional capture methods to provide greater confidence that the eradication was successful (e.g. Davison et al., 2017). As *G. affinis* can naturally disperse from the introduced population, and it is also used as a biological control agent as well as an ornamental species (CABI), so new introductions are likely to occur after an eradication if public awareness and education do not accompany the management work (Bremner and Park, 2007).	
	Drainage of ponds combined with biomanipulation (e.g. use of native predators)	The eradication strategy consists of a combination of the drainage of ponds with *G. affinis* presence, possibly accompanied by lime treatment, and biomanipulation. Both actions are best combined, but in some cases can be applied solely. Draining requires complete control over drainage and refill of the water body. To reduce the impact on other native fish, the native species are caught and kept in quarantine after draining to be restocked on site after refill (e.g. Britton and Brazier, 2006). Care is taken to check these lots of fish before restocking to prevent reintroduction of any remaining mosquitofish. As small mosquitofish easily get stuck in the nets	High

		biosecurity measures are applied to clean this gear before moving to another location. In ponds with native amphibians present, drainage should be performed between September and January, after metamorphosis of the amphibian larvae and before the start of the new breeding season, with the timing of the eradication procedure such that it avoids or minimises the risks of non-target organisms (invertebrates, amphibians) being affected by the work. Biomanipulation involves stocking water bodies with **native** predatory fish, notably juvenile northern pike (*Esox lucius*) or large Eurasian perch (*Perca fluviatilis*; see Davison et al. 2017). This technique appears to be effective for topmouth gudgeon, with in some cases complete eradication of topmouth gudgeon and almost no reduction in native species (Lemmens et al. 2015). Ideally, depending on the local situation, this is a combination of pelagic predators such as Eurasian perch and littoral predators such as northern pike, because juvenile pike tend to reside in the littoral vegetation and ambush prey fishes as they swim past the vegetation. To increase the resilience to new invasions, water bodies where mosquitofish have been removed, it may be appropriate and advisable to stock in native predatory fish as aftercare. The cost of the removal and temporary keeping in containers of the native fishes, the draining of the pond and the consequent stocking with predatory fish is not very high (< €100k; the estimated cost is for the removal and temporary keeping. Although the cost of removal may vary according to surface area, the cost of quarantine is less likely to do so, i.e. a larger water body may contain fewer non-target fish than a smaller water body). However, this method can only be applied when the distribution of the mosquitofish is restricted to ponds.	
Methods to achieve management [6]	**Raising awareness**: Raising public awareness of the risks posed by invasive alien species in general and	Costs for outreach and production of leaflets can be high when applied across a large community, such as the EU.	Medium

	G. affinis in particular. The production of targeted publicity and identification material.		
	The methods described to support eradication can also be used to manage existing *Gambusia* populations.	See above	See above
	Reducing risks of further dispersal	Dedicated monitoring of water courses and water bodies is necessary to detect the presence of *G. affinis* and to ensure they are not recolonised by the species after eradication e.g. electrofishing, fyke nets but also eDNA. In parallel, to prevent further spread and new introductions, a prohibition on the keeping (as an aquarium fish), release (for mosquito control) and use of the species as live bait should be enforced. Also, stringent procedures should be put in place to check imported and within-EU consignments of fish intended for stocking, both into fish farms and into open waters, for contamination with small-bodied non-native species, such as *Gambusia* spp., topmouth gudgeon and fathead minnow (*Pimphales promelas*). Depending on the area that has to be monitored, management costs can be from medium to very high (from <€5k to > €1m). Small ponds are biodiversity hot spots, containing proportionally more species than rivers and lakes. So, a small investment (e.g. €5k) to management a small pond will have disproportionately higher positive impact for native biodiversity. Whereas, a larger water body may be the only location where an invasive species exists, so a higher cost is needed to achieve a similar benefit to native biodiversity, i.e. through elimination of the potential source population for neighbouring invasions.	Medium

Cited literature

Allen, Y., Kirby, S., Copp, G.H. & Brazier, M. 2006. Toxicity of rotenone to topmouth gudgeon *Pseudorasbora parva* for the species' eradication from a tarn in Cumbria. Fisheries Management & Ecology 13: 337–340.

Bremner, A. & Park, K. 2007. Public attitudes to the management of invasive non-native species in Scotland. Biological Conservation 139, 306–314.

Britton, J.R. & Brazier M. 2006. Eradicating the invasive topmouth gudgeon, *Pseudorasbora parva*, from a recreational fishery in northern England. Fisheries Management and Ecology 13, 329–335.

Britton, J.R., Davies, G.D. & Brazier, M. 2010. Towards the successful control of the invasive *Pseudorasbora parva* in the UK. Biological Invasions 12, 125–131.

Davison, P.I., Copp, G.H., Créach, V., Vilizzi, L. & Britton, J.R. 2017. Application of environmental DNA analysis to inform invasive fish eradication operations. Science of Nature 104, 35.

Lemmens, P., Mergeay, J., Vanhove, T., De Meester, L. & Declerck, S.A.J. 2015. Suppression of invasive topmouth gudgeon *Pseudorasbora parva* by native pike *Esox lucius* in ponds. Aquatic Conservation: Marine and Freshwater Ecosystems 25, 41–48.

Roy, H.E., Rorke, S.L., Beckmann, B., Booy, O., Botham, M.S., Brown, P.M., Harrower, C., Noble, D., Sewell, J. & Walker, K. 2015. The contribution of volunteer recorders to our understanding of biological invasions. Biological Journal of the Linnean Society of London, 115, 678–689.

Weyl, O.L.F., Ellender, B.R., Woodford, D.J. & Jordaan, M.S. 2013. Fish distributions in the Rondegat River, Cape Floristic Region, South Africa, and the immediate impact of rotenone treatment in an invaded reach. African Journal of Aquatic Science 38, 201–209.