

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2019/812602/ETU/ENV.D.2¹

Name of organism: *Misgurnus anguillicaudatus* (Cantor, 1842)

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

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¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: The present risk assessment covers one species (no lower taxa): *Misgurnus anguillicaudatus* (Cantor, 1842). It belongs to the genus *Misgurnus* Lacepède 1803, which is part of the Cobitidae (loaches or weatherfishes) (Froese and Pauly 2023).

Actinopterygii > Cypriniformes (Carps) > Cobitidae (Loaches or weatherfishes) > Cobitinae > *Misgurnus* Lacepède 1803 > *Misgurnus anguillicaudatus* (Cantor, 1842)

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii (ray-finned fishes)

Order: Cypriniformes (Carps)

Family: Cobitidae (Loaches)

Genus: *Misgurnus* Lacepède 1803

Cobitidae consists of 28 genera and 262 species (Froese and Pauly 2023); but the taxonomy is not fully clear and the number of recognised species differs from study to study and database to database and is still subject to new research.

Recently, Eschmeyer's Catalog of Fishes (Fricke et al. 2023), reintegrated the genus *Paramisgurnus* within the genus *Misgurnus*. Following Fricke et al. (2023), the genus *Misgurnus* now contains 12 valid species:

Misgurnus amamianus Nakajima & Hashiguchi 2022

Misgurnus anguillicaudatus (Cantor 1842)

Misgurnus bipartitus (Sauvage and Dabry de Thiersant 1874)

Misgurnus buphoensis Kim and Park 1995

Misgurnus chipisaniensis Shedko & Vasil'eva 2022

Misgurnus fossilis (Linnaeus 1758)

Misgurnus mohoity (Dybowski 1869)

Misgurnus multimaculatus Rendahl 1944

Misgurnus nahangensis (Nguyen & Bui 2009)

Misgurnus nikolskyi Vasil'eva 2001

Misgurnus tonkinensis Rendahl 1937

Misgurnus dabryanus (Guichenot 1872)

The latter species was only recently renamed from *Paramisgurnus dabryanus* Dabry de Thiersant, 1872 to *Misgurnus dabryanus* (Guichenot 1872) (Fricke et al. 2023).

Zangl et al. (2020) state that the specimens of *M. anguillicaudatus* in Austria (and parts of Germany) were misidentified and should be *M. bipartitus* (based on morphology and genetics) instead. Genetic data from INBO (Brys et al. 2020) point in the same direction and conclude that the non-native weatherfish occurring in Belgium (and the Netherlands) is not *M. anguillicaudatus* but indeed *M. bipartitus*.

According to Kottelat and Freyhof (2007), only two non-native 'oriental' weatherfish were recorded in the risk assessment (RA) area (*M. anguillicaudatus* and *M. dabryanus*). However, Zięba et al. (2010) report that a fish imported under the name *Misgurnus anguillicaudatus* in the UK was found to be *M. mizolepis*. This reproducing loach population in a pond in Essex is since eradicated (Zięba et al. 2010). *M. bipartitus* is the fourth Asian or oriental weatherfish of the genus *Misgurnus* in the RA area (Brys et al. 2020, Zangl et al. 2020).

The three Asian or oriental weatherfish species that have been established in Europe to date, *M. bipartitus*, *M. anguillicaudatus* and *M. dabryanus*, can usually be easily distinguished from the European weatherfish *M. fossilis* by the lack of the characteristic longitudinal stripes of the latter species, which are also easily recognisable in juvenile animals. In contrast to *M. fossilis*, the males of the non-native species develop a lamina circularis at the base of the pectoral fins. *M. dabryanus* differs from the other two non-native species by its large body size, the pronounced fat ridge on the caudal peduncle, the larger scales and the absence of a black spot at the base of the caudal fin. This black dot is clearly pronounced in *M. bipartitus*, but is also absent in *M. fossilis* (Jung et al. 2021).

Yi et al. (2017) made a morphological comparison and DNA barcoding of four closely related *Misgurnus* species to evaluate the feasibility of morphological and genetic identification of the species *M. dabryanus*, *M. anguillicaudatus*, *M. bipartitus* and *M. mohoity*. Twelve morphological characters were measured in 542 individuals to perform the comparative analysis. Among these characters, only the caudal peduncle length revealed significant difference ($P < 0.05$) among these four species.

Li et al. (2010) measured eleven morphometrical items to evaluate the morphological variations in 'Amur' weatherfish *M. mohoity*, 'northern oriental' weatherfish *M. bipartitus* and 'oriental' weatherfish *M. anguillicaudatus* by three methods of multivariate analysis. Cluster analysis showed that 'northern oriental' weatherfish was close to 'oriental' weatherfish, and distantly related to 'Amur' weatherfish in genetic relationship (Li et al. 2010). Population genetic studies by Yi et al. (2016a, 2018) corroborate this close relationship between *M. anguillicaudatus* and *M. bipartitus*.

Vasil'eva (2001) gives the following morphological characteristics for the differentiation between *M. anguillicaudatus* and *M. bipartitus*:

The length of the caudal peduncle is 1.1-1.9 times the height of the caudal peduncle, the distance between the base of the ventral fin and the base of the anal fin is 1.3-1.4 times the distance between the base of the anal fin and the base of the caudal fin, 43 – 47 vertebrae => *M. anguillicaudatus*

The length of the caudal peduncle is 1.9-2.9 times the height of the caudal peduncle, the distance between the base of the ventral fin and the base of the anal fin is 2.0-2.5 times the distance between the base of the anal fin and the base of the caudal fin, 48 – 51 vertebrae => *M. bipartitus*.

The preferred common name in English is oriental weatherfish, but also dojo, Japanese weatherfish, pond loach and Asian weather loach are used as common names. Scientific (non-valid) synonyms for *M. anguillicaudatus* following FishBase (Froese and Pauly 2023) are *Cobitis anguillicaudata*, *Misgurnus anguillicaudatus anguillicaudatus*, *Misgurnus fossilis anguillicaudatus*, *Misgurnus aguillicadatus*, *Nemachilus lividus*, *Misgurnus lividus*, *Nemacheilus lividus*, *Misgurnus crossochilus*, *Ussuria leptcephala*, *Misgurnus mizolepis grangeri*, *Misgurnus mohoity leopardus*, *Misgurnus mohoity yunnan*, *Misgurnus punctatus*, *Misgurnus mizolepis punctatus*, *Misgurnus mizolepis unicolor*, *Misgurnus elongatus*, *Misgurnus mizolepis elongatus*, *Cobitis fossilis*, *Cobitis fossilis mohoity*, *Misgurnus mohoity*, *Misgurnus mizolepis*, *Misgurnus mizolepis multimaculatus*.

In the RA area, *M. anguillicaudatus* (oriental weatherfish) currently probably is established in four countries. Populations are reported from The Netherlands (van Kessel et al. 2013a; Niemeijer and van Kessel 2016), Germany (Freyhof and Korte 2005), Spain (Franch et al. 2008, Clavero et al. 2023), and Italy (Razzetti et al. 2001). Possibly some of these populations are misidentified and are in fact *M. bipartitus* (see Zangl et al. 2020); confirmed populations of *M. bipartitus* exist in Austria and Germany (Zangl et al. 2020), Belgium and The Netherlands (Brys et al. 2020). In France, two records of *M. anguillicaudatus* are known from the Seine basin (N. Poulet, pers. com.) but the species is not established in this Member State. Recently, Clavero et al. (2023) have found established populations of *M. anguillicaudatus* and of hybrids between *M. anguillicaudatus* and *M. bipartitus* in Catalonia (Spain). Other, non-established Asian weatherfish species are reported from Switzerland and Germany, namely *M. dabryanus* (large-scale loach) (Freyhof and Brooks 2011; Stoeckle et al. 2019) and from the UK, *M. mizolepis* (Zięba et al. 2010).

Misgurnus anguillicaudatus is known to hybridise naturally with *M. dabryanus* (You et al. 2009). In aquaculture, Fujimoto et al. (2008a) were able to produce hybrids of *M. anguillicaudatus* and *M. mizolepis*. Artificial reproduction was also used to successfully crossbreed *M. anguillicaudatus* with the native *M. fossilis* (J. Wanzenböck, pers. comm.). Recently, hybrids between *M. anguillicaudatus* and *M. bipartitus* have been found in Catalonia (Clavero et al. 2023). Hybrids between these two species are explicitly included in this risk assessment, as it has to be assumed that impacts are identical to the parental species.

The known common names of *M. anguillicaudatus* in European languages other than English are the following: FR: loche asiatique, IT: cobite di stagno orientale, DK: kinesisk smerling, DE: Ostasiatischer Schlammpeitzger, NL: Aziatische modderkruiper, SLO: azijska činklja.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: Loaches and weatherfishes from the genus *Misgurnus* have a long eel-like body and ten chin barbels. The Global Invasive Species Database (GISD; <http://www.iucngisd.org/gisd/species.php?sc=1537>) describes *Misgurnus anguillicaudatus* as “brown to yellow with greenish, gray-brown to black marbling, with a paler ventral. Its eel-like body is long, laterally compressed, commonly measuring around 15 cm, but reaching a maximum size near 30 cm. It has a small, narrow mouth and subinferior with 10 barbels, 4 of them, clearly smaller than the other, placed below the lower lip. Its lips are thick and fleshy. Its lateral line is short and does not exceed the length of the pectoral fin. Its pectoral fins are triangular with a stout spine. The dorsal fin originates above the pelvic fin origin and is single and short-based. It bears conspicuous adipose crests along the ventral and dorsal mid-lines of the caudal peduncle. It also has a characteristic dark spot in the upper half of the base of the caudal fin. It has 9 dorsal rays, 6-7 pelvic rays, and 7-8 anal rays. Generally males have larger pectoral fins and females fuller abdomens”. Males can be distinguished from females (sexual dimorphism) by the enlarged second ray of the pectoral fin and the presence (vs. absence in females) of the lamina circularis (Urquhart and Koetsier 2011).

Kottelat and Freyhof (2007) state the *M. anguillicaudatus* can be distinguished from other species of *Misgurnus* in Europe by (1) dark, irregular spots on the body and no stripes, (2) the caudal peduncle has low adipose crests, (3) lamina circularis (i.e. ventral base of the second pectoral ray broadens into a rounded plate and covers the bases of the other pectoral rays) is present in males and (4) there is a dark spot on the upper caudal base.

In the RA area, only one species of the genus *Misgurnus* is native (*Misgurnus fossilis*). Its native range encompasses Europe north of the Alps, from the Meuse eastward to the Neva drainages and Lake Ladoga, the northern Black Sea basin from the Danube eastward to Kuban and the Caspian basin in the Volga and Ural drainages (Kottelat and Freyhof 2007). It is not native in Great Britain, Scandinavia and Iberia. The European weatherfish *M. fossilis* also has an anguilliform body shape and ten barbels but has a broad midlateral line from eye to caudal base and a narrow stripe from opercle at least to pelvic origin instead of irregular spots on the body for *M. anguillicaudatus*. A lamina circularis is absent in males while it is present in *M. anguillicaudatus* (Kottelat and Freyhof 2007). Other native loaches belong to separate genera (*Cobitis* and *Sabanejewia*) and can be readily distinguished from *Misgurnus* because the suborbital spine is externally visible (*versus* hidden under muscles in *Misgurnus*) and the lower lip has 0-2 short, barbel-like mental lobes (vs. 4 long, barbel-like mental lobes in *Misgurnus*) (Kottelat and Freyhof, 2007).

Discussed below are other non-native *Misgurnus* species that can be potential substitute species.

Misgurnus bipartitus is morphologically very similar to *M. anguillicaudatus*, but good identification keys are not available (Frable 2008). Yi et al. (2017) provide a morphological comparison of four Chinese *Misgurnus* species (*M. dabryanus*, *M. anguillicaudatus*, *M. bipartitus*, *M. mohoity*) and conclude that morphometric measurements can be used to distinguish between these species but also warn that “efficient delimitation of these four closely species based on one or several morphological

characters seems to be difficult and limited". Population genetic studies by Yi et al. (2017, 2018), however, showed that these genetic analysis can be used as an efficient identifier of the different Chinese weatherfish species.

Zangl et al. (2020) based their morphological identification of *M. bipartitus* on characters given in the key by Vasil'eva (2001 and the translated Chinese key therein) namely the caudal peduncle depth 2.4–2.5 times in caudal peduncle length vs. 1.3–1.8 times in *M. anguillicaudatus* and the maximum body depth 8.2–8.6 times in standard length (SL) vs. < 7.5 times in *M. anguillicaudatus*. The latter species is more elongated (Nichols 1943).

The Chinese fine-scaled loach *M. mizolepis* is closely related to *M. anguillicaudatus*. This species can be readily distinguished from the oriental weatherfish because it has a greater number of fine surface scales covering its body rather than irregularly embedded scales (Frale 2008). Moreover the peduncular keels (adipose crests near caudal peduncle) are greatly developed in *M. mizolepis* while they are low in *M. anguillicaudatus* (Nichols 1943; Kottelat and Freyhof 2007).

As with *M. mizolepis* the adipose crests near the caudal peduncle are greatly developed in the large-scale loach *M. dabryanus*. This species can be distinguished from *M. anguillicaudatus* because the dark dot on the upper caudal base is missing and from *M. fossilis* by the absence of a broad midlateral stripe from eye to caudal base, and the absence of the narrow stripe from opercle to the pelvic origin (Stoeckle et al. 2019).

As systematics and taxonomy are still not fully resolved in the genus *Misgurnus* correct identification of *Misgurnus* species remains difficult (Zangl et al. 2020). Many authors, however, are able to correctly identify, morphologically as well as genetically, the different oriental weatherfish species (Yi et al. 2016b, 2017, 2018; Zangl et al. 2020, Jung et al. 2021).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: A risk assessment was performed for South Australia in 2014 (Fredberg et al. 2014). The authors used FISK and NAPRA tools to assess, rank and score the environmental, economic and social impacts and costs of *M. anguillicaudatus* and found that the species has a high risk of establishment and several potential impacts were suggested, including competition for spawning sites and for food and shelter, disturbance and/or predation of fish and frog eggs, alteration of habitat and decreased water quality. Fredberg et al. (2014), however, concluded that these impacts were largely speculative and further research is needed to fill several knowledge gaps.

For the USA, an Ecological Risk Screening Summary was written by The U.S. Fish and Wildlife Service in 2012. *Misgurnus anguillicaudatus* is established in the US and they concluded that there is a high risk of additional introductions, establishment, and impacts in other areas because this species is highly adaptable, quick to reproduce, and is extremely popular in the aquaculture and aquarium industry. The climate match with the native range is high and multiple impacts are expected (reductions in macroinvertebrate populations, altered aquatic habitats, and the fish are vectors for certain fish parasites). Their overall risk was estimated as high but with a medium certainty of the assessment.

Interesova et al. (2020) assessed a number of non-native fishes in the River Ob Basin (Russia) and rated *M. anguillicaudatus* as having a high risk of becoming invasive. The species was also assessed for state of New York and the New York Invasiveness Rank was High (http://nyis.info/wp-content/uploads/2017/10/1ae76_Misgurnus-anguillicaudatus-Ecological.pdf).

In the Netherlands, Spikmans et al. (2010) made a pest risk analysis for ten non-native fish species based on literature and climate match. For oriental weatherfish they found a high current climate match with most of the Dutch rivers suitable for establishment of this fish. They do not assess the invasiveness of this species but only summarise the literature on possible impacts (predation, competition and disease transmission).

Almeida et al. (2013), using FISK for the Iberian Peninsula, and Radočaj et al. (2021), using AS-ISK for Croatia and Slovenia, both report high scores for the invasiveness of *M. anguillicaudatus*.

All the above risk assessments are valid for the RA area because of a reasonable to good climate match and the high adaptability of the oriental weatherloach.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: The oriental weatherfish is native to the East Asian temperate regions, including Cambodia, China, India, Japan, Korea, Laos, Taiwan, Thailand, Vietnam and the Tugur and Amur River catchments in Siberia (Kottelat and Freyhof 2007). In its native habitat, *M. anguillicaudatus* is found in mangrove swamps along rivers and lakes. In addition, the species is found in ponds, marshes and rice fields (Spikmans et al. 2010). In general, the species prefers stagnant or weakly flowing water (Froese and Pauly 2023). The oriental weatherfish is mainly found in waters with a muddy bottom. The species burrows in sediment or leaf litter, with only its head protruding (Simon et al. 2006, Froese and Pauly 2023). Natural spread from its native area into the risk assessment area is not possible.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Outside the RA area, oriental weatherfish was introduced to Australia (Koster et al. 2002 (extensive overview of Australian populations), Keller and Lake 2007, Fredberg et al. 2014), North America (several authors, e.g. Tabor et al. 2001, Simon et al. 2006), South America (Abilhoa et al. 2013) and Asia outside of their natural range (Belle et al. 2017).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given

separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): **Continental** (Freyhof and Korte 2005; Belle et al. 2017), **Mediterranean** (Franch et al. 2008; Razzetti et al. 2001, Clavero et al. 2023). All references are very trustworthy and report the first records first hand. Although van Kessel et al. (2013a) report *M. anguillicaudatus* from the **Atlantic** region, this species turned out to be *M. bipartitus* after a genetic screening (Brys et al. 2020).

Response (6b): **Continental** (Freyhof and Korte (2005) report adults and juveniles and suggest that a successfully reproducing population has been established in Germany), **Mediterranean** (Franch et al. (2008) state that oriental weatherfish has an established population in the Ebro, northeastern Iberian Peninsula). Recently, Clavero et al. (2023) confirmed the establishment of *M. anguillicaudatus* and a hybrid of *M. anguillicaudatus* and *M. bipartitus* in Catalonia (Spain). All references are very trustworthy and report the first records first hand. Natural reproduction and presence of young fishes was reported for The Netherlands (Atlantic region) by Binnendijk et al. (2017), however, this species turned out to be *M. bipartitus* after a genetic screening (Brys et al. 2020).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate

² Convention on Biological Diversity, Decision VI/23

change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): The species could currently establish in all EU biogeographic regions (Annex VIII). The highest suitability is in the Black Sea, Continental, Mediterranean and Pannonian regions followed from high to low by the Steppic, Boreal, Atlantic and Alpine regions (see figure 1).

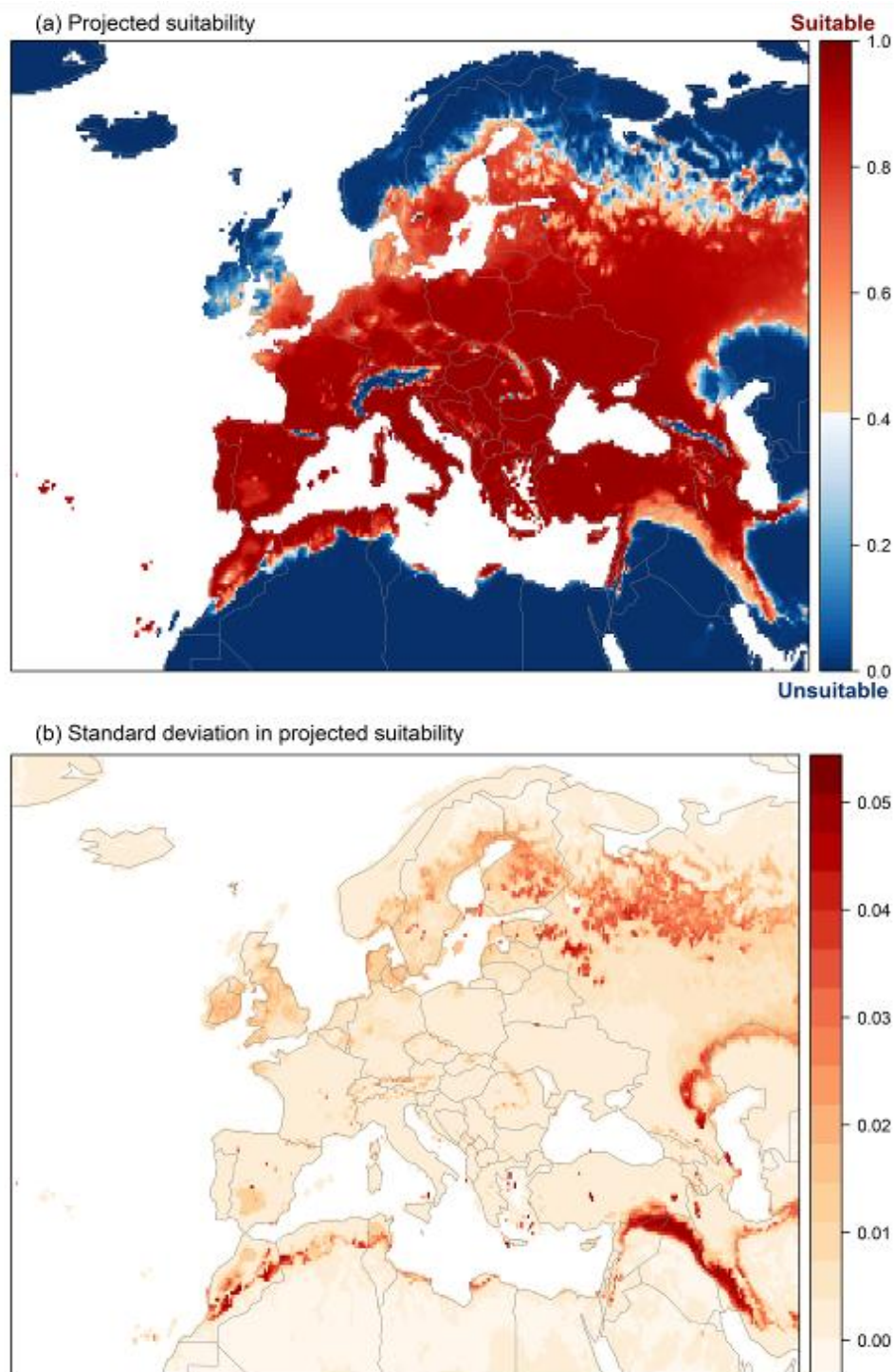


Figure 1: (a) Projected current suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

Response (7b): The species could establish in the 2070s under climate change scenario RCP2.6 and scenario RCP4.5 in all EU biogeographic regions (Annex VIII). The highest suitability is in the Black

Sea, Continental, Mediterranean and Pannonian regions followed from high to low by the Steppic, Atlantic, Boreal and Alpine regions (see figure 2 for scenario RCP2.6).

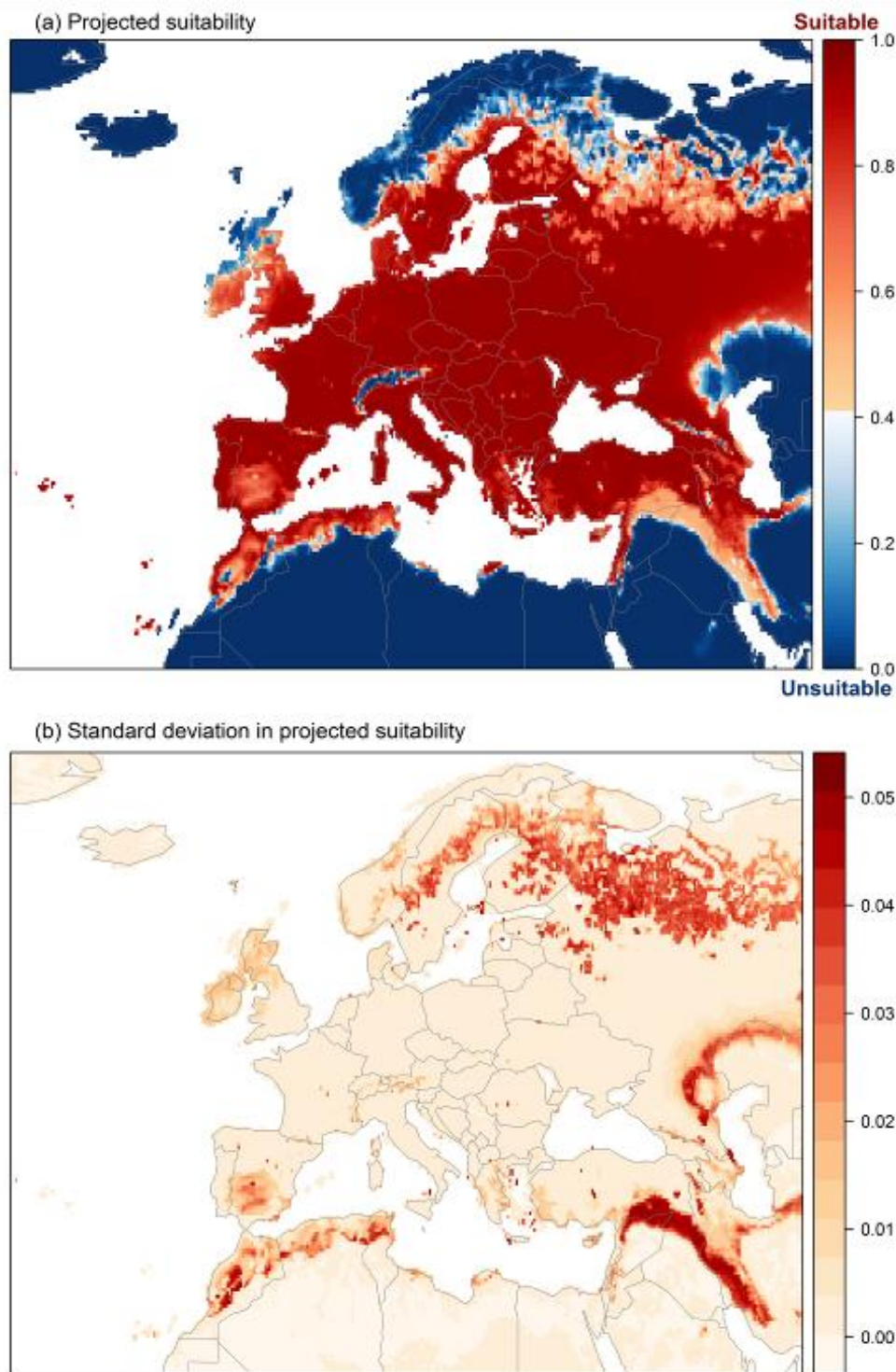


Figure 2: (a) Projected suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 1. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

It is not totally clear which aspects of climate change are most likely to affect the risk assessment but the SDM (Annex VIII) suggested that suitability for *Misgurnus anguillicaudatus* was strongly determined by the maximum temperature of the warmest month (27.1%) and by annual precipitation (23.8%). In both climate change scenarios, the suitable area will increase in the colder parts of the RA area e.g. UK, Ireland, Finland and Sweden.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs to be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): The oriental weatherfish was first recorded in Germany in 1990 (Freyhof and Korte 2005). Subsequently first records came from Italy in 1997 (Razzetti et al. 2001), Spain in 2001 (Franch et al. 2008) and the Netherlands in 2012 (van Kessel et al. 2013a).

It must be noted that possibly some of these populations are misidentified and in fact are other *Misgurnus* species. Recently it became clear, after genetic screening, that confirmed populations of *M. bipartitus* exist in Austria and Germany (Zangl et al. 2020), and Belgium and the Netherlands (Brys et al. 2020). It is, however, impossible to check all the reported *M. anguillicaudatus* specimens on their correct identification.

Response (8b): After the first record in 1990 in Germany (Freyhof and Korte 2005), in 2002, 43 specimens were caught ranging from 5.5 to 20.0 cm standard length; these specimens included adults and juveniles, suggesting a successfully reproducing, established population. Some specimens were caught in 2016 in the River Inn (South Germany), but establishment is not confirmed (Belle et al. 2017).

Razzetti et al. (2001) reported the first record for Italy in 1997, more individuals were caught in 1998 and the presence of dozens of adults and young fish of both sexes (total length between 9 and 21 cm) in 2000 confirmed the establishment of this species there.

Since the first Spanish record in 2001, the population of oriental weatherfish is expanding (Franch et al. 2008). The capture of over 1,000 weatherfish shows that its population is composed of both juvenile and adult individuals suggesting an established population. Recently, Clavero et al. (2023) confirmed the establishment of *M. anguillicaudatus* and a hybrid of *M. anguillicaudatus* and *M. bipartitus* in Catalonia (Spain).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

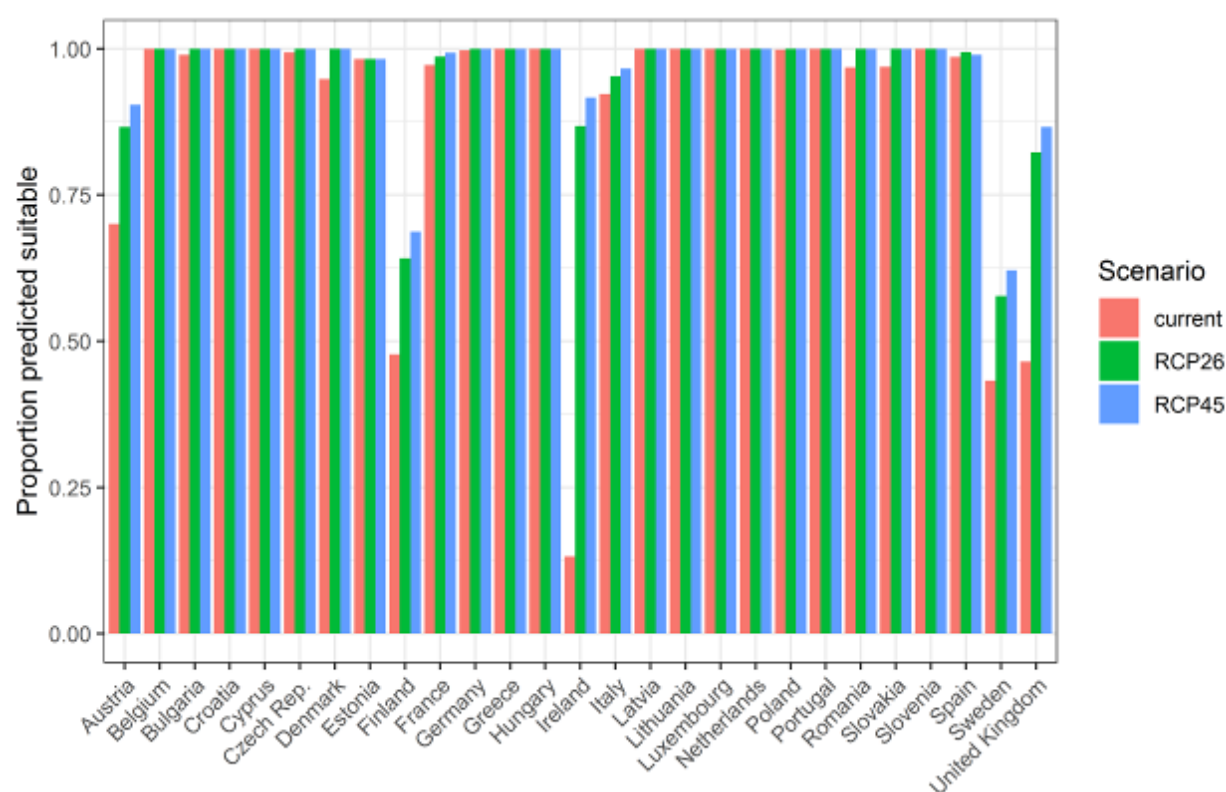
With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Under current climate conditions *M. anguillicaudatus* could establish in all EU MS and the UK; there seems to be a high establishment potential for most MS but less so for Ireland, UK, Sweden, Finland and Austria (see figure 3).



Response (9b): In the 2070s, under climate change scenario RCP2.6 and scenario RCP4.5 *M. anguillicaudatus* could establish in all EU MS and the UK: there seems to be a high establishment potential for most MS with increased potential (compared to current conditions) for Ireland, UK, Sweden, Finland and Austria (see Figure 3).

It is not totally clear which aspects of climate change are most likely to affect the risk assessment but the SDM (Annex VIII) suggested that suitability for *Misgurnus anguillicaudatus* was strongly determined by the maximum temperature of the warmest month (27.1%) and by annual precipitation (23.8%). In both climate change scenarios, the suitable area for establishment will increase in the colder parts of the RA area e.g. UK, Ireland, Finland and Sweden.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: Several authors reported adverse impacts but most of these impacts are anecdotal. For instance, Nobile et al. (2017) sum up possible impacts of established oriental weatherfishes for Brazil as follows: niche overlap, competition for resources and space, introduction of parasites, predation on eggs of fishes and restructuring of the aquatic ecosystem.

Keller and Lake (2007) report the potential impacts for Australia. In a mesocosm experiment, they found that oriental weatherfish caused significant reductions in macroinvertebrate numbers and biomass and that this fish was also associated with elevated ammonia, nitrate/nitrite (NO_x), and turbidity levels. However, the authors conclude: “We are not able to conclusively determine that oriental weatherfish are having large impacts in natural systems”.

In Asia, the species is known to hybridize with other species of *Misgurnus* (You et al. 2009; Fujimoto et al. 2008a), but it is not clear what the impact of this phenomenon will be.

Schmidt and Schmidt (2014) conclude that *M. anguillicaudatus* does not seem to affect other vertebrates in the Hudson River Valley (New York) but that attention should be paid on the potential negative interactions (competition for food) with the native eastern mudminnow *Umbra pygmaea*. Similarly, López et al. (2012) suggest potential competition for food resources with benthic species such as river blenny (*Salaria fluviatilis*), European eel (*Anguilla anguilla*) and Iberian loach (*Cobitis paludica*). Also, Radočaj et al. (2021) state that in Croatia and Slovenia, *M. anguillicaudatus* can produce hybrids with congeneric the native *M. fossilis* with impacts on their genetic diversity

Misgurnus anguillicaudatus has been responsible for the establishment of the parasitic flatworm *Gyrodactylus macracanthus* (Dove and Ernst 1998) in Australia. Ogawa (1994) names this loach as host of the gill parasite *Ancyrocephalus cruciatus*, fin parasite *Gyrodactylus micracanthus*, and other unidentified fin and gill parasites of the genus *Gyrodactylus*. *Misgurnus anguillicaudatus* can also host the parasite *Acanthocephalus opsariichthydis* (Amin et al. 2007). In addition, the gill parasites *Trichodina lechriodontata* and *Trichodina modesta* were found in oriental weatherfish (Zhao and Tang 2007). Also Reyda et al. (2019) report the introduction of at least three *Gyrodactylus* species in the USA through oriental weatherfish. These parasites can potentially infect indigenous fish species and can cause mortalities in aquaculture as was the case with another *Gyrodactylus* parasite (*G. salaris*) in Norway (Salte et al. 2010).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Established populations in the RA area are reported in the Continental (Freyhof and Korte 2005) and Mediterranean biogeographic regions (Ebro delta (Franch et al. 2008) and the province of Pavia (Razzetti et al. 2001)). López et al. (2012) state densities of up to 100 individuals m⁻² of sampled area. Although it is not always explicitly stated in the papers that report the establishment of self-sustaining populations, most authors mention potential negative impacts that imply invasiveness (because of growing numbers and expanding populations).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: Invasiveness is not explicitly stated in the papers that report established, self-sustaining populations in the RA area. Most impacts mentioned are anecdotal but imply invasiveness. In the future, invasiveness may be expected (because of growing numbers and expanding populations) in Germany (central east near Frankfurt am Main), Italy (Po river basin), and Spain (northeast, Ebro delta). Regarding, the Ebro population (northeastern Iberia) this population is highly abundant and its control is no longer possible. According to López et al. (2012) this species has already spread to other drainages within the northeastern part of Iberia. In the Netherlands (southeastern part near Weert) and Belgium (northeastern part near Bocholt), the congener *M. bipartitus* is showing an explosive population growth in just a few years (author's own observation).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.

- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: *Misgurnus anguillicaudatus* is sold as an ornamental fish in the aquarium trade in the RA area as well as in the rest of the world (Strecker et al. 2011; Chang et al. 2009; Franch et al. 2008; Tabor et al. 2001), as a food source (Park et al. 2006) and as live bait (Franch et al. 2008). However, no published figures on quantities and costs were found.

In China (and other Asian countries), oriental weatherfish is a highly valued food fish and also used in traditional Chinese medicine. Since the 1990s the entire life cycle of this fish can be produced in aquaculture facilities and as of 2010 more than 204.552 tons were produced in aquaculture in China (Milton et al. 2018).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Two main pathways are identified:

Pathway name: Escape from confinement – Pet/Aquarium/Terrarium species

This pathway is estimated to be the most important for introduction and entry of the oriental weatherfish in the RA area, with the species being available for sale on the internet⁵ and in shops. Disposal of aquarium fishes and escape into the wild from ponds happens frequently (Chan et al. 2019).

Pathway name: Escape from confinement – Live food and live bait

Although no data are available on the use of oriental weatherfish as live food or live bait in the RA area; it is an important food source in its native range, especially in China. So it can be assumed that this fish is intentionally imported to e.g. Chinatowns in Europe. The use of this species as live bait in the RA area is unclear and only mentioned as a possible introduction route by Franch et al. (2008). In Australia as well as in the USA and Japan, Koster et al. (2002) confirmed the use of this species as live bait.

Pathway: Escape from confinement – Pet/Aquarium/Terrarium species

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: *Misgurnus anguillicaudatus* has been imported into the RA area as an aquarium and garden pond species, it still is sold in many fish shops and on the internet. Entry into the wild was probably both intentional (release of unwanted specimens) and unintentional (flooding of garden ponds).

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

⁵ <https://www.justcichlids.com/index.php/pages/Dojo-loach-for-sale.html>;
<https://verduijncichlids.com/product/misgurnus-anguillicaudatus/>

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The aquarium trade is responsible for the worldwide movement of over 2 billion live freshwater fish per year (Chan et al. 2019). In Australia ornamental trade has become the leading vector for non-native fish introductions since the 1970s with species established in Australian drainages including oriental weatherfish. Oriental weatherfish were imported to Australia by the aquarium industry at a rate of up to 50,000 per year until the sale was banned in 1986 (Keller and Lake 2007). For the RA area, no exact data are available but several authors (e.g. van Kessel et al. 2013a, Zangl et al. 2020) mention this pathway (“aquarium trade”) as the most likely for some Member States. Although the propagule pressure of introductions into the RA area may be high, the entry of the introduced fishes into the wild will be much lower. Still, releases of aquarium fish occur frequently (Chan et al. 2019). No information has been found on the percentage of introduced fishes for ornamental purposes in the risk assessment area or entering the natural environment.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Live transport of aquarium species is well organised, the survival during the passage would be high as with other fish transports. Moreover, oriental weatherfish is a hardy fish that can withstand reduced dissolved oxygen conditions (Koetsien and Urquhart 2012) and low temperatures (Urquhart and Koetsier 2014). Reproduction during transport is unlikely but not impossible because reproduction as well as fertilization of the eggs happens in the water column. Eggs hatch within 30 hours and larvae remain in the water column for a short period before settling on the bottom (Frale 2008). Survival of specimens that get released in the wild (dumped from aquaria or escaped after flooding) is bound to be

high because of the hardiness of oriental weatherfish and its ability to survive several days out of the water (Koster et al. 2002).

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Intentional fish transport is meant to keep the fish alive, so no management practices to imperil the survival would be in place. Aquarium holders will do their best to keep their fish alive under the best conditions and no management practices would be in place to prevent entry and survival in the wild.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Misgurnus* species can be difficult to distinguish from each other for non-trained people, e.g. *M. anguillicaudatus* imported for the aquarium trade in Great Britain turned out to be *M. mizolepis* instead (Zięba et al. 2010), the same can happen the other way round. Escape into the wild of aquarium and pond fishes happens frequently and may be noticed only years later (Chan et al. 2019). *M. anguillicaudatus* can be detected during regular monitoring for e.g. the Water Framework Directive but there may be several years between first occurrence and first detection. Also, this species has a benthic lifestyle and often lives in marshlike habitats which are difficult to sample. New detection methods, e.g. environmental DNA (eDNA) may be used for better and quicker detection of this cryptic species (Brys et al. 2020).

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Introductions via the aquarium trade can happen in any place, especially since *M. anguillicaudatus* is also for sale through the internet⁶. Intentional as well as unintentional entry into the wild can also occur in most places but propagule pressure is bound to be higher in a neighbourhood with dense human populations (Copp et al. 2005).

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The oriental weatherfish is already established in several Member States (MS) of the RA area as a consequence of releases/escapes connected to the aquarium trade (Zangl et al. 2020). Additional introductions are still possible and probably ongoing since this species is readily available via the aquarium trade and the internet. Entry into the wild has already been encountered for several decades now, with natural populations in at least six MS (NL, BE, DE, AT, ES, IT).

Pathway: Escape from confinement – Live food and live bait

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: As this fish is highly valued as a food source (especially in China), intentional import of this species to e.g. Chinatowns in the RA area cannot be excluded. The import of oriental weatherfish for use as live bait is not documented for the RA area but was for Australia, the USA and Japan (Koster et al. 2002).

⁶ <https://verduijnclids.com/product/misgurnus-anguillicaudatus/>
<https://www.gdaquarium.nl/a-28161018/tropische-vissen-database/misgurnus-anguillicaudatus-gold-weeraal-goud/#description>

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Although no information has been found on organised imports of live weatherfish for food or as baits into the RA area, it is still possible that this species, farmed in large numbers in Asia, is intentionally imported to e.g. Chinatowns in Europe. Several authors (Franch et al. 2008, Milton et al. 2018) report that *M. anguillicaudatus* is important as a food source, especially in China (and some other East Asian countries). The use of this species in the RA area as live bait is unclear and only mentioned as a possible introduction route by Franch et al. (2008) but was confirmed for Australia, the USA and Japan (Koster et al. 2002).

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Live transport of food species is well organised, the survival during the passage would be high as with other fish transports. Moreover, oriental weatherfish is a hardy fish that can withstand reduced dissolved oxygen conditions (Koetsien and Urquhart 2012) and low temperatures (Urquhart and Koetsier 2014). Reproduction during the transport is unlikely but not impossible because

reproduction as well as fertilization of the eggs happens in the water column. Eggs hatch within 30 hours and larvae remain in water column for a short period before settling on the bottom (Frable 2008). Survival of specimens that get released in the wild (e.g. release of excess live bait) is expected to be high because of the hardiness of oriental weatherfish and its ability to survive several days out of the water (Koster et al. 2002).

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Intentional fish transport is meant to keep the fish alive, so no management practices to imperil the survival would be in place. Neither would management practices be in place to prevent survival of escaped fish in the wild.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Misgurnus* species can be difficult to distinguish from each other for non-trained people, e.g. *M. anguillicaudatus* imported for the aquarium trade in Great Britain turned out to be *M. mizolepis* instead (Zięba et al. 2010), the same can happen the other way round. Escape into the wild of live food fishes happens frequently and may be noticed only years later (Rixon et al. 2005).

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Introductions for the live food trade or as live bait can happen in any place, especially since *M. anguillicaudatus* is also for sale through the internet. Although no information has been found on organised import of live weatherfish for food into the RA area, it is possible that this species is imported in Asian neighbourhoods of cities in Europe. Intentional as well as unintentional entry into wild can occur in these places where propagule pressure is higher near the neighbourhood of live food markets, but these live food markets are probably not widespread in the RA area.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Importation of live fishes is known as an important pathway for the introduction of invasive, non-native fishes in some regions in the world (Rixon et al. 2005), but probably less so in the RA area. However no specific information has been found on this subject.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The oriental weatherfish is already established in several Member States of the RA area. Additional introductions are still possible and probably ongoing since this species is readily available via the aquarium trade and the internet. It is also farmed in high numbers in China for the food industry. Several MS have already reported established populations in the wild as a consequence of deliberate introductions from aquaria or unintentional escapes from garden ponds.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided.

However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is established from NW Europe, over Central Europe to the south in Spain and Italy. The risk of introduction and/or entry is very likely and will probably not change much in foreseeable climate change conditions. Possibly northern MS will be at higher risk of invasion in the future because the temperature range of *M. anguillicaudatus* is very broad, this can be assumed from its broad native habitat. It is not clear which aspects of climate change are most likely to affect the risk assessment but an increase in average winter temperature will increase the suitable area in the colder parts of the RA area e.g. Great Britain, Ireland, Finland and Sweden (Annex VIII).

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Oriental weatherfish is a fish native mainly to the temperate climate region of east Asia (Köppen-Geiger climate classification) (Kottelat and Freyhof 2007). All temperate regions in the world (e.g. USA, Australia, Europe) have reported established populations of this species. In the RA area, the species is established from Central Europe (Germany) to the south in Spain and Italy. Probably all MS can contain established populations of *M. anguillicaudatus*, because it is a very hardy species that can tolerate harsh conditions like reduced dissolved oxygen conditions (Koetsien and Urquhart 2012) and low temperatures (Urquhart and Koetsier 2014). Specimens of this fish or its congener *M. bipartitus* have already been found in a cold alpine stretch of the River Inn in Germany and Austria (Zangl et al. 2020) but it is not known whether they are established there. Figure 1 (at page 11) shows a high (projected) suitability for establishment in most of the EU MS.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE		CONFIDENCE	
	very isolated		low
	isolated		medium
	moderately widespread		high
	widespread		
	ubiquitous		

Response: *M. anguillicaudatus* occurs in rivers, lakes and ponds (Froese and Pauly 2023) and also in swamps and ricefields (You et al. 2009). It prefers muddy bottoms, where they hide in the muck and leaf litter with only their heads sticking out. This species is very hardy and can survive a wide range of temperatures and environmental conditions. It is typically found in slow or still waters with muddy or silty bottoms abundant with aquatic plants (Froese and Pauly 2023). However, Schmidt and Schmidt (2014) noticed that their sample sites in the Hudson Valley (NY, USA) were mostly devoid of aquatic plants. Several authors (e.g. Franch et al. 2008; Zangl et al. 2020) mention also rivers as suitable habitat for the oriental weatherfish. The oriental weatherfish can breathe atmospheric oxygen by using its intestine as an accessory respiratory organ, allowing it to live in oxygen-poor waters and to bury itself in soft substrates to survive long droughts (Koetsier and Urquhart 2012). All these suitable habitats are widely available in the RA area.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Misgurnus anguillicaudatus* is established in the RA area in a wide range of habitats and climate conditions (see Q 2.1) where also other species occur. The congener species, *M. bipartitus*, for instance co-exists, in very high densities, with *M. fossilis* and *Cobitis taenia* (spined loach), two species with a similar habitat use and food items in its Dutch and Belgian non-native range (Brys et al. 2020). and *M. bipartitus* occurs there. In the Dutch population, top predators like pike *Esox lucius* and perch *Perca fluviatilis* were present in the fish community (Binnendijk et al. 2017) and not limiting establishment of *M. bipartitus*. Similar situations can be expected for the close relative *M. anguillicaudatus*.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As in other parts of the world (USA, Australia, Brazil), *M. anguillicaudatus* became established in several regions in the RA area despite the presence of native predators (e.g. northern

pike *Esox lucius* and Eurasian perch *Perca fluviatilis*), parasites or pathogens (Koster et al. 2002, Binnendijk et al. 2017).

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Currently, no management practices seem to be ongoing in the RA area, no information has been found on this subject. Previous efforts to eradicate (or at least manage) the Dutch population (Binnendijk et al. 2017) failed. Over several years and multiple times per year Binnendijk et al. (2017) tried to catch (with dipnets and electrofishing) as many loaches as possible and removed them from the infested site. Although many specimens were captured (n= 763), eradication efforts were stopped because the numbers kept increasing with new and smaller (i.e. natural reproduction) caught each year. Other eradication methods were deemed unfeasible on the basis of financial, practical, ecological or legal reasons (Binnendijk et al. 2017). The Dutch management effort is the only management practice reported for the RA area.

In the context of European, national and regional legislations (e.g. Decision on the free migration of fish species in the hydrographic catchment areas of the Benelux countries⁷) several actions are undertaken to remove fish migration barriers from rivers and streams. These management practices to restore the natural connections may actually facilitate spread and enhance establishment of non-native species to new areas (Krieg and Zenker 2020). However, in the case of the oriental weatherfish, weirs and dams may have been less of a barrier for dispersal because Lintermans et al. (1990) reported observations of this species moving overland.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

⁷ https://www.vmm.be/wetgeving/M_2009_1_NL.pdf

Response: The oriental weatherfish can breathe atmospheric oxygen by using its intestine as an accessory respiratory organ, allowing it to live in oxygen-poor waters and to bury itself in soft substrates to survive long droughts (Koetsier and Urquhart 2012). Eradication campaigns for fish often use the principle of water removal (e.g. drying of ponds) or oxygen depletion (e.g. use of piscicides) to kill unwanted fish species. Because of its unique feature to breathe atmospheric oxygen, oriental weatherfish is able to survive oxygen deprived conditions for many days. In experimental circumstances in the USA, some individuals survived for over 81 days in desiccated conditions with soil moisture content less than 3% (Koetsier and Urquhart 2014). An effort to eradicate (or at least manage) the Dutch *M. bipartitus* population, by using multiple catches and removals of individuals with electrofishing apparatus, fykes and dipnets failed (Binnendijk et al. 2017). On the other hand, a reproducing population of *M. mizolepis* in a small, plastic-lined garden pond in Essex has been eradicated (Zięba et al. 2010).

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Misgurnus anguillicaudatus* matures quickly, can reproduce multiple times in a season, and produces a great number of offspring (Nico et al. 2020). The male wraps his body around the female and stimulates her to release a cloud of eggs, which he simultaneously fertilizes. These fish are nonguarders and release their eggs in open water as well as on substratum (Breder and Rosen 1966). This indicates broad environmental conditions for reproduction. Yamamoto and Tagawa (2000) reported 2000 eggs per female for introduced weatherfish in Hawaii. However, Urquhart (2013) found an estimated number of mature ova in female fish ranged from zero up to 46,18 for a self-sustaining population in Idaho (USA). Within this study area, oriental weatherfish lived 5–7 years, reaching sexual maturity within the first year of life. Females remained fecund throughout their lifetime, and spawned twice per year, with young produced in the late spring (May) and early autumn (October) seasons.

The oriental weatherfish is also a very hardy species with broad temperature tolerances (Urquhart and Koetsier 2014) and can withstand low dissolved oxygen conditions and dessication (Koetsier and Urquhart 2012).

No data are available about the genetic diversity of the oriental weatherfish populations in the RA area. Belcik (2017) investigated the population genetics of introduced oriental weatherfish populations in the Chicago Area Waterways and concluded that the results from his study suggest a single introduction to these waterways before 1987, with a subsequent range expansion. This author also suggests that the population is genetically identical to the weatherfish introduced into Australia before 1984, and that these populations were derived from the same native population in Asia. Therefore, it seems fair to conclude that low genetic diversity of this species probably will not inhibit its ability to establish in new areas. Binnendijk et al. (2017) also assume a single introduction in the southeast of the Netherlands was responsible for the establishment for a large population of *M. bipartitus*.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Most MS contain suitable habitat for oriental weatherfish to survive and inhabit, even if establishment would not be possible, there is a continued chance of e.g. aquarium specimens being dumped (Chan et al. 2019) as there is a continued sale of this species for the aquarium trade throughout the RA area and they will survive because of their broad tolerance for physiological variables, a flexible diet and a high reproductive potential (Koetsier and Urquhart 2012).

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response: *M. anguillicaudatus* is established in several MS in the RA area (Spain, Italy, Germany; maybe also the Netherlands). The SDM (Annex VIII) pointed out that most MS and most biogeographical regions have favourable conditions for establishment. Suitable habitats are available all over the RA area and because of its flexibility and broad tolerance for physiological variables, new introductions of oriental weatherfish will almost certainly lead to new established populations. Moreover, indications exist that oriental weatherfish can reproduce by gynogenesis (Morishima et al. 2002), a form of asexual reproduction in which growth and development of embryos occur without fertilization and where the egg are stimulated to develop simply by the presence of sperm: in the case of *M. anguillicaudatus* the sperm of a cyprinid species e.g. gibel carp *Carassius gibelio* is sufficient. This means that even the escape into the wild of one mature female can be enough to start a population. Also, there may be several years between first occurrence and first detection as *M. anguillicaudatus* has a benthic lifestyle and often lives in marshlike habitats which are difficult to sample with traditional methods like electrofishing and fykes. New detection methods, e.g. environmental DNA (eDNA) may be used for better and quicker detection of this cryptic species (Brys et al. 2020).

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species could establish in the 2070s under climate change scenario RCP2.6 in all EU biogeographic regions (Annex VIII). The highest calculated suitability is in the Black Sea, Continental, Mediterranean and Pannonian regions followed from high to low by the Steppic, Atlantic, Boreal and Alpine regions (see figure 2). Under scenario RCP4.5, conditions get better for most ecoregions in the RA area (figure 4). The SDM (Annex VIII) suggested that suitability for *Misgurnus anguillicaudatus* was strongly determined by the maximum temperature of the warmest month (27.1%), followed by annual precipitation (23.8%) while minimum temperature of the coldest month only accounted for 8.8% of the variation.

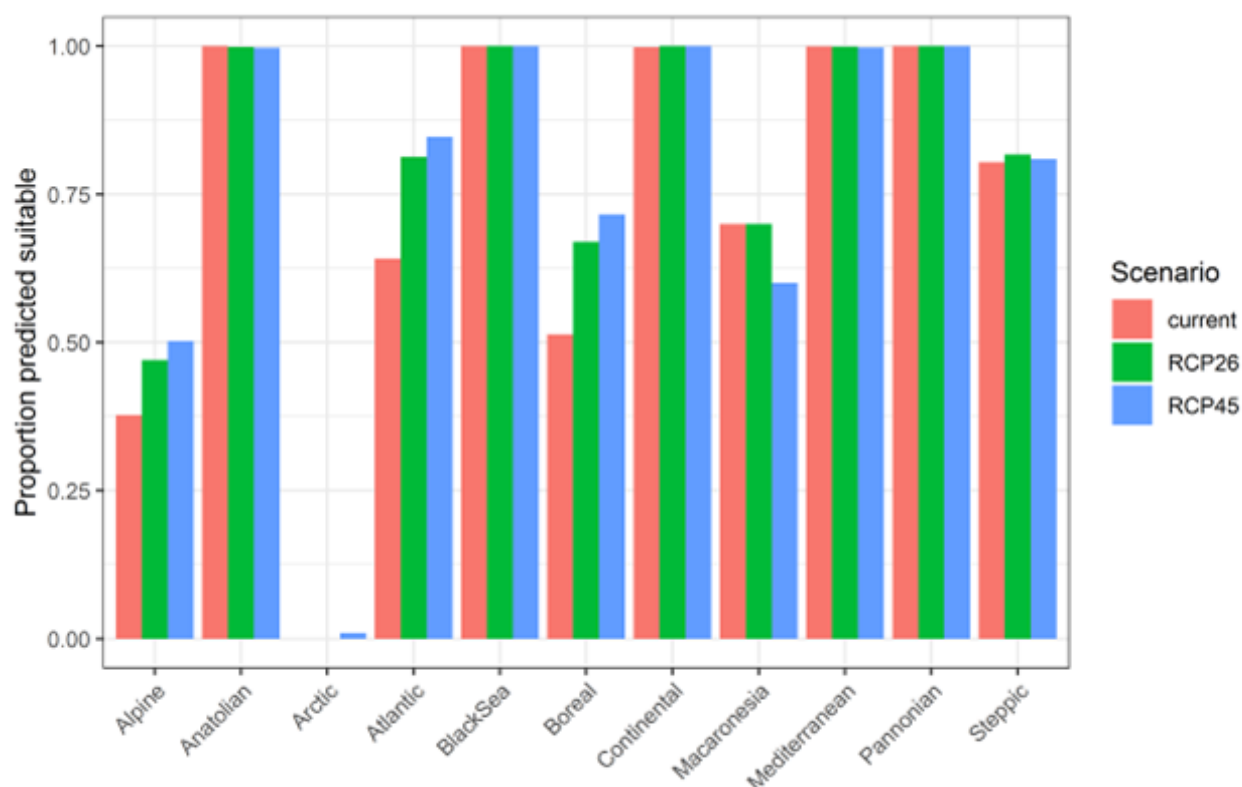


Figure 4: Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among biogeographical regions of Europe. The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Anatolian, Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: Natural spread can occur between connecting waters. Although overland movements are theoretically possible because of its air breathing capacity, Koetsier and Urquhart (2012) concluded that even in worsening environmental conditions (dessication experiments) oriental weatherfish rather bury themselves in the mud than trying to move away. However, Koster et al. (2002) reported the find of a specimen of oriental weatherfish moving overland during a wet day a short distance outside of a garden pond in Australia. Schmidt and Schmidt (2014) report that oriental weatherfish had colonized at least 20 km of the Dwaar Kill mainstem (plus several tributaries) and it is present in at least 8.5 km of the Wallkill River; this spread was probably realised over the last 20-30 years.

In the RA area, in the Ebro delta, oriental weatherfish was first detected in 2001 and by 2007 the species had spread over 31 1 x 1 km UTM quadrates (Franch et al. 2008). López et al. (2012) state that this species has large capacity to disperse from the established populations, finally colonizing all the Ebro delta.

In the Netherlands, natural spread of the congener *M. bipartitus* was also relatively high; from the discovery in 2012 to the end of 2016, this oriental weatherfish had moved twelve kilometers downstream and one kilometer (and possibly more) upstream (Binnendijk et al. 2017). In the downstream direction it looks like the spread was stopped by a (temporary) barrier. The Belgian

population originated from natural spread from the neighbouring Dutch population, through a natural connection, with seven years between first records in the Netherlands and Belgium (Brys et al. 2020).

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except "Unaided (Natural Spread)", which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Only one pathway for spread was identified: Corridor – Interconnected waterways / basins / seas. All over the introduced range, secondary spread was noticed from initial infection sources. In the RA area most river catchments are connected by man-made canals, which act as corridors for the spread of invasive aquatic species (Leuven et al. 2009), however, it is unclear how important this pathway is for *M. anguillicaudatus* in the RA area.

Pathway name: Corridor – Interconnected waterways / basins / seas

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: In the RA area most river catchments are connected by man-made canals, which act as corridors for the spread of invasive aquatic species (Leuven et al. 2009). Although the canals are intentionally built to connect water basins to allow boat traffic between them, the spread through these systems is unintentional.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Range expansion of the oriental weatherfish through man-made channels and canals was not investigated in the RA area. However, in the USA, oriental weatherfish used the North Shore Channel and the Chicago Sanitary and Ship Canal to move from the Lake Michigan Basin side toward the rivers and tributaries of the Mississippi River Basin (Belcik 2017). In Australia, these fish have also been collected from concrete flood control channels, and rock-filled wire cages used as soil conservation structures (Koster et al. 2002). In the native range, oriental weatherfish are known to inhabit irrigation channels for rice fields (Fujimoto et al. 2008b). The above indicates that channels and canals are valid corridors for the dispersal of the oriental weatherfish.

No numbers are available to give an indication of the propagule pressure through this pathway. However, a few specimens are enough to start a new population (e.g. Dutch and German population: van Kessel et al. 2013a, Freyhof and Korte 2005). On top of this, indications exist that oriental weatherfish can reproduce by gynogenesis (Morishima et al. 2002).

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: NA

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Currently, no management practices seem to be ongoing in the RA area, no information has been found on this subject. Previous efforts to eradicate (or at least manage) the Dutch population (Binnendijk et al. 2017) failed. Over several years and multiple times per year Binnendijk et al. (2017) tried to catch (with dipnets and electrofishing) as many loaches as possible and removed them from the infested site. Although many specimens were captured (n= 763), eradication efforts were stopped because the numbers kept increasing with new and smaller young-of-the-year individuals (i.e. natural reproduction) caught each year. Other eradication methods were deemed unfeasible on the basis of financial, practical, ecological or legal reasons (Binnendijk et al. 2017). Chemicals (e.g. rotenone) can be used to eradicate fish in small, closed waters but are very difficult to apply in large, open water systems like canals. Also are these chemicals not species-specific and cause high collateral damage among non-target species. The use of these chemicals is also legally restricted (Koster et al. 2002).

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No information has been found on dedicated monitoring of oriental weatherfish in the RA area. *M. anguillicaudatus* can be detected during regular monitoring for e.g. the Water Framework Directive but there may be several years between first occurrence and first detection. Also, this species has a benthic lifestyle and often lives in marshlike habitats which are difficult to sample. New detection methods, e.g. environmental DNA (eDNA) may be used for better and quicker detection of this cryptic species (Brys et al. 2020).

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Much of the RA area offers suitable habitat for oriental weatherfish. The corridors provide a perfect pathway between the suitable habitats in different river catchments. There are no data about the presence of oriental weatherfish in canals in the RA area. In other introduced areas e.g. Belcik (2017) reported that the oriental weatherfish used the North Shore Channel and the Chicago Sanitary and Ship Canal to move from the Lake Michigan Basin side toward the rivers and tributaries of the Mississippi River Basin.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: No information has been found on the spread potential through this pathway in the RA area, but considering the moderate expected spread by natural means and the estimated relatively low number of existing populations of oriental weatherfish in the RA area, the rate of spread based on this pathway is thought to be moderate even though environmental conditions are favourable in most MS of the RA area.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: Natural spread is very difficult to contain. In the RA area most river catchments are connected by artificial canals. Once oriental weatherfish is introduced, it will spread almost unnoticed, until one or a few specimens are coincidentally detected. Containment of this species in large river and other natural water systems is almost impossible, in common with most fish species. In smaller systems oriental weatherfish may be contained using piscicides (e.g. rotenone) but with high collateral damage. Also, piscicides may have legal restrictions of its use in several Member States of the RA area.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Oriental weatherloach do not appear to undertake migrations over long distances as part of their life-cycle (Lucas and Barras 2001). However, little appears to be known about their general movement patterns (Koster et al. 2002). As indicated in Q 3.1, the rate of spread in most countries and continents is not very fast (probably less than 10 km per year). A study by Schultz (1960) determined that in the Shiawassee River in Michigan, their expansion was slow, around 0.8 km per year but a study conducted in Australia concluded that oriental weatherfish can expand their range more quickly at about 7.2 km per year (Lintermans et al. 1990). Frable (2008) states that within just two years, oriental weatherfish populations had spread more than 35km in Washington. Koster et al. (2002) report that oriental weatherfish may be restricted in their ability to colonise in an upstream direction.

Currently there are only a few known established populations of oriental weatherfish in the RA area and, despite the high availability of suitable habitat, it is estimated that the overall potential rate of spread under current conditions is rather slow. The spread via corridors (e.g. canals) and through natural waterways is probably only of importance for Member States/regions with neighbouring existing populations in the RA area.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Although the establishment potential is expected to increase in the foreseeable climate change conditions (see Qs A7 and A9), the rate of spread rate is not expected to change compared to current conditions.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU+UK, excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- ##

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: Fredberg et al. (2014) suggest several potential impacts of oriental weatherfish in Australia including competition for spawning sites and for food and shelter, disturbance and/or predation of fish and frog eggs, alteration of habitat and decreased water quality. They, however, concluded that these impacts were largely speculative and further research is needed to fill several knowledge gaps.

For the USA, the U.S. Fish and Wildlife Service (2012) reported that there is a high risk of impacts because this species is highly adaptable, quick to reproduce, and is extremely popular in the aquaculture and aquarium industry. The climate match with the native range is high and multiple impacts are expected (reductions in macroinvertebrate populations, altered aquatic habitats, and the fish are vectors for certain fish parasites).

Several authors reported adverse impacts but most of these impacts are anecdotal. For instance, Nobile et al. (2017) sum up possible impacts of established oriental weatherfishes for Brazil as follows: niche overlap, competition for resources and space, introduction of parasites, predation on eggs of fishes and restructuration of the aquatic ecosystem.

Keller and Lake (2007) report the potential impacts for Australia. In a mesocosm experiment, they found that oriental weatherfish caused significant reductions in macroinvertebrate numbers and biomass and that this fish was also associated with elevated ammonia, nitrate/nitrite (NO_x), and turbidity levels. However, the authors conclude: “We are not able to conclusively determine that oriental weatherfish are having large impacts in natural systems”.

In Asia, the species is known to hybridize with other species of *Misgurnus* (You et al. 2009; Fujimoto et al. 2008a) but it is not clear what the impact of this phenomenon will be. *Misgurnus anguillicaudatus* is known to hybridise naturally with *M. dabryanus* (You et al. 2009). In aquaculture, Fujimoto et al. (2008a) were able to produce hybrids of *M. anguillicaudatus* and *M. mizolepis*.

Schmidt and Schmidt (2014) conclude that *M. anguillicaudatus* does not seem to affect other vertebrates in the Hudson River Valley (New York) but that attention should be paid on the potential negative interactions with the native eastern mudminnow *Umbra pygmaea*.

M. anguillicaudatus has been responsible for the establishment of the parasitic flatworm *Gyrodactylus macracanthus* (Dove and Ernst 1998) in Australia. Ogawa (1994) names this loach as host of the gill parasite *Ancyrocephalus cruciatus*, fin parasite *Gyrodactylus micracanthus*, and other unidentified fin and gill parasites of the genus *Gyrodactylus*. *Gyrodactylus* species can potentially infect indigenous fish species and can cause mortalities in aquaculture as was the case with another *Gyrodactylus* parasite (*G. salaris*) in Norway (Salte et al. 2010). *M. anguillicaudatus* can also host the parasite *Acanthocephalus opsariichthydis* (Amin et al. 2007). In addition, the gill parasites *Trichodina lechriodentata* and *Trichodina modesta* were found in oriental weatherfish (Zhao and Tang 2007). Also Reyda et al. (2019) report the introduction of at least three *Gyrodactylus* species in the USA through oriental weatherfish.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: No studies were found that explicitly investigate the impact in the RA area. Within the RA area, the same biodiversity impacts as in Qu 4.1., however, can be assumed. Of major concern is the fact that *M. anguillicaudatus* is known to hybridise naturally with other weatherfishes e.g. with *M. dabryanus* (You et al. 2009). Artificial reproduction was used to successfully crossbreed *M. anguillicaudatus* with the native *M. fossilis* (J. Wanzenböck, pers. comm.). The European weatherfish *M. fossilis* is highly endangered in parts of its native territory, e.g. Belgium (Verreycken et al. 2014) with populations declining everywhere in the EU (Freyhof and Brooks 2011). Co-occurrence of a close relative of the oriental weatherfish, *M. bipartitus*, with European weatherfish was found in sites in the Netherlands and in Belgium and because *M. bipartitus* occurs in much larger numbers than the rare European weatherfish, the latter is automatically exposed to a much greater hybridization pressure (Brys et al. 2020).

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Probably the current potential impact will not change much in the near future. However, if *M. anguillicaudatus* spreads further and more oriental weatherfish populations would establish then the potential impact might increase. Especially in areas where *M. fossilis* populations are declining, the relative pressure of *M. anguillicaudatus* on this native species will be higher in the future. Also when higher densities of oriental weatherfish occur, there could be other impacts, for example alteration of macroinvertebrate communities and decline of water quality in lakes and streams (Keller and Lake 2007).

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor	CONFIDENCE	low medium
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	moderate major massive		high
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Response: The European weatherfish is protected by EU law (Habitats Directive Annex II and Bern Convention Annex III). It is listed as a species of least concern but as declining everywhere in the native range on the IUCN Red List of Threatened Species (Freyhof and Brooks 2011). Hartvich et al. (2010) report the conservation status of *M. fossilis* as follows: Endangered on the Red List in the Czech Republic; Danger of extinction, category 1 in Austria; Vulnerable in Croatia, Near Threatened in Slovakia and Vulnerable in Poland. The species is also listed as Endangered on the Red List of Flanders (Belgium) (Verreycken et al. 2014) and as Vulnerable on the Red List of The Netherlands (Spikmans and Kranenburg 2016). The goal to preserve and improve populations of *M. fossilis* in the EU can be seriously hampered by the presence of oriental weatherfish through possible competition and hybridization. Probably no other native species are impacted directly by the oriental weatherfish as there are no closely related species present in the RA area.

The impact of oriental weatherfish on protected sites in the RA area (e.g. Natura 2000 habitats or particular habitats listed in the Habitats Directive) is not clear. However, as oriental weatherfish was associated with elevated ammonia, nitrate/nitrite (NO_x), and turbidity levels in mesocosm experiments in Australia (Keller and Lake 2007), the impact on these protected habitats may become moderate to major should high densities of oriental weatherfish establish there.

Also the ecological status of water bodies according to the Water Framework Directive (WFD) can be lower due to the presence of non-native species. Some groups (e.g. habitat-sensitive) fish species are of great importance for the WFD assessment of certain water types. Water managers assess the ecological status of waters, among other things, on the relative share of "habitat sensitive" species in the WFD assessment. The negative effects on these species as a result of competition with non-native species may have a negative effect on the results of WFD assessments (van Kessel et al. 2013b).

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No major changes are expected for the future. However, should *M. anguillicaudatus* further expand its spread and establish in more sites and at higher densities, then slightly higher impacts as mentioned in Qu. 4.4. can be expected.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: No information has been found on the issue but three ecosystem services impacts can be possible in a minor form (1) Provisioning/Genetic material/ Genetic material from animals i.e. possible loss of local genetic material of *M. fossilis* as a consequence of hybridisation with oriental weatherfish (Brys et al. 2020) and (2) Regulations and Maintenance/Regulation of physical/chemical/biological condition/Water condition through elevated ammonia, nitrate/nitrite (NOx), and turbidity levels (Keller and Lake 2007) and (3) Regulations and Maintenance/Pest and Disease control when harmful parasites are introduced (Reyda et al. 2019).

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A	CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: No information has been found on the issue.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	N/A	CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: No information has been found on the issue.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: No economic costs (e.g. management) of this species are mentioned. Established populations of oriental weatherfish are not actively managed in any state or territory in Australia, nor at a national level (Koster et al. 2002) but eradication with rotenone was trialled in the Wingecarribee River system and was unsuccessful. Eradication campaigns with rotenone are known to be costly. In the USA, rotenone treatments of large areas to remove (but not completely eradicate) common carps and ictalurid catfishes were reported to cost between US\$25,000 for 31 ha (\approx €730/ha) and US\$40,000 for 492 ha (\approx €74/ha) (U.S. Fish and Wildlife Service - Environmental Conservation Online System, 2020). In Hawaii, the control of tilapia with rotenone (CFT Legumine) was estimated to cost US\$5000 for 81 ha (€56/ha) (only product was counted, not personnel and equipment) (Tavares 2009).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No economic costs of this species are known.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No economic costs of this species are known for the current nor future situation.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal	CONFIDENCE	low
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	minor moderate major massive		medium high
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Response: The only reported management programme of oriental weatherfish in the RA area is for the Netherlands. Efforts to eradicate (or at least manage) the Dutch population (Binnendijk et al. 2017) failed. Over several years and multiple times per year Binnendijk et al. (2017) tried to catch (with dipnets and electrofishing) as many loaches as possible and removed them from the infested site. Although many specimens were captured (n= 763), eradication efforts were stopped because the numbers kept increasing with new and smaller young-of-the-year individuals (i.e. natural reproduction) caught each year. The costs of these management efforts were not reported. Other eradication methods were deemed unfeasible on the basis of financial, practical, ecological or legal reasons (Binnendijk et al. 2017).

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Currently no targeted management schemes exist for oriental weatherfish in the RA area. In the past, only one management programme of oriental weatherfish in the RA area was reported (i.e. for the Netherlands (Binnendijk et al. 2017)). However, if *M. anguillicaudatus* were to further spread and establish large populations, then management costs would increase with expanding populations.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;

- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue and although no information on social and human health impacts is available for the RA area, it can be assumed that this is only minimal since reports from other (longer) invaded areas (e.g. Australia and the USA) do not explicitly mention these impacts either (Frable 2008, Koster et al. 2002).

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue but see previous answer.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Misgurnus anguillicaudatus* has been responsible for the establishment of the parasitic flatworm *Gyrodactylus macracanthus* (Dove and Ernst 1998) in Australia. Ogawa (1994) names this loach as host of the gill parasite *Ancyrocephalus cruciatus*, fin parasite *Gyrodactylus micracanthus*, and other unidentified fin and gill parasites of the genus *Gyrodactylus*. *Misgurnus anguillicaudatus* can also host the parasite *Acanthocephalus opsariichthydis* (Amin et al. 2007). In addition, also the gill parasites *Trichodina lechriodentata* and *Trichodina modesta* were found in oriental weatherfish (Zhao and Tang 2007). Reyda et al. (2019) report the introduction of at least three *Gyrodactylus* species in the USA through oriental weatherfish. These parasites can potentially infect indigenous fish species and can cause mortalities in aquaculture as was the case with another *Gyrodactylus* parasite (*G. salaris*) in Norway (Salte et al. 2010).

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A	CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: No information has been found on the issue.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: The main impact of oriental weatherfish could be the possible hybridisation with the native, endangered European weatherfish *M. fossilis* (Brys et al. 2020). However, it can also indirectly affect native species through competition (e.g. for food (Schmidt and Schmidt 2014) and may affect the aquatic environment by modifying conditions such as altering water quality (Keller and Lake 2007). The natural control of the populations of oriental weatherfish by other organisms may be limited but their role is mostly unknown. Though there have been occasional reports of oriental weatherfish being eaten by redfin perch and brown trout, they often occur in shallow degraded habitats which large native predators may avoid (Koster et al. 2002). In the Dutch population of *M. bipartitus*, top predators

like pike *Esox lucius* and perch *Perca fluviatilis* were present in the fish community (Binnendijk et al. 2017) and not limiting the establishment of this invasive species.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The major concern of *M. anguillicaudatus* is the potential hybridisation with the native *M. fossilis*. The European weatherfish is highly endangered in parts of its native territory with populations declining everywhere in the EU (Freyhof and Brooks 2013). Co-occurrence of oriental weatherfish with European weatherfish can expose a great hybridization pressure on the latter (Brys et al. 2020). Oriental weatherfish can also indirectly affect native species through competition and may affect the aquatic environment by modifying conditions such as altering water quality and uprooting plants (Keller and Lake 2007). *Misgurnus anguillicaudatus* is also identified as carrier of several parasites and some of these (e.g. *Gyrodactylus* species) may be harmful to wild populations of native fish and to the aquaculture industry.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No large changes in impact are to be expected in future climate conditions although some biogeographical regions (see Qu. A7) may have a higher establishment suitability. Little is known about the overall impact in the risk assessment area in the current situation (see Qu. 4.19), however, in the future, the overall impact may increase if more oriental weatherfish populations become established.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The oriental weatherfish is present in several member states (MS) of the RA area. Additional introductions are possible and probably ongoing since this species is available via the aquarium trade and the internet. It is also farmed in high numbers in China for the food industry.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	Several MS have established populations in the wild as a consequence of deliberate introductions and unintentional escapes. The species could currently establish in all EU biogeographic regions and conditions improve for most regions under climate change.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	The rate of spread is not very fast (probably less than 10 km per year). Currently there are only a few known established populations in the RA area and despite the high availability of suitable habitat it is estimated that the overall potential rate of spread under current and future conditions is only moderate.
Summarise Impact*	minimal minor moderate major massive	low medium high	The major concern of <i>M. anguillicaudatus</i> is the potential hybridisation with the endangered native <i>M. fossilis</i> . Oriental weatherfish can also indirectly affect native species through competition and may affect the aquatic environment by modifying conditions such as altering water quality and uprooting plants and carry several parasites which may be harmful to wild populations of native fish and to the aquaculture industry. Future impacts are mostly unknown but may increase if more populations of oriental weatherfish were to establish.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	Since the risks of introduction and entry and of establishment are high (with high confidence) and impacts are moderate (with medium confidence), the overall

			risk is high. Specifically, the threat to the endangered native European weatherfish <i>M. fossilis</i> is of great concern.
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*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

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

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	?	?	Yes	Yes	?
Belgium	?	?	Yes	Yes	?
Bulgaria	–	–	Yes	Yes	–
Croatia	–	–	Yes	Yes	–
Cyprus	–	–	Yes	Yes	–
Czech Republic	–	–	Yes	Yes	–
Denmark	–	–	Yes	Yes	–
Estonia	–	–	Yes	Yes	–
Finland	–	–	?	Yes	–
France	–	–	Yes	Yes	–
Germany	Yes	Yes	Yes	Yes	Yes
Greece	–	–	Yes	Yes	–
Hungary	–	–	Yes	Yes	–
Ireland	–	–	?	Yes	–
Italy	Yes	Yes	Yes	Yes	Yes
Latvia	–	–	Yes	Yes	–
Lithuania	–	–	Yes	Yes	–
Luxembourg	–	–	Yes	Yes	–
Malta	–	–	Yes	Yes	–
Netherlands	?	?	Yes	Yes	Yes
Poland	–	–	Yes	Yes	–
Portugal	–	–	Yes	Yes	–
Romania	–	–	Yes	Yes	–
Slovakia	–	–	Yes	Yes	–
Slovenia	–	–	Yes	Yes	–
Spain	Yes	Yes	Yes	Yes	Yes
Sweden	–	–	?	Yes	–

Biogeographical regions of the risk assessment area

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

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 occurred somewhere at least once in the last millenium	1 in 1,000 years
Possible	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	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, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁸	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 reversible; 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.

⁸ Not to be confused with "no impact".

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

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 <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> 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 <i>and/or</i> 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 <i>and/or</i> 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) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



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

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

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

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u> <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water⁹	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction</u> ; <u>noise attenuation</u> ; <u>visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection

⁹ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

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

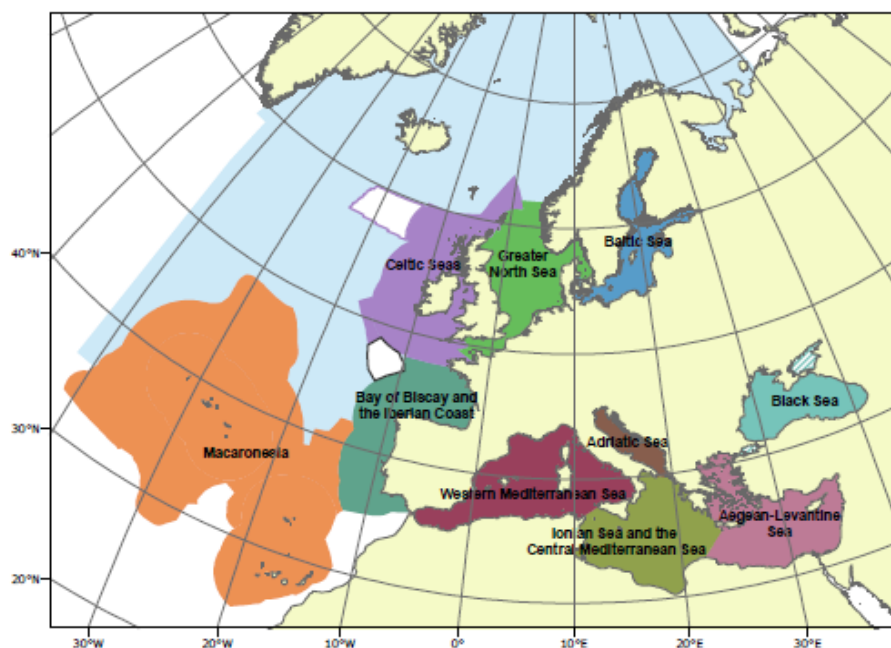
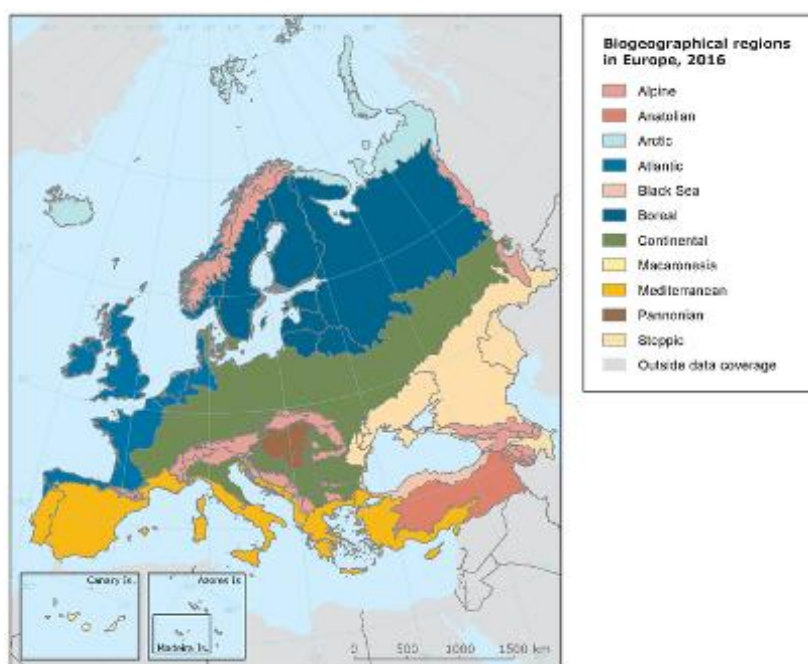
			<p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>
	<p>Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting</p>	<p>Spiritual, symbolic and other interactions with natural environment</p>	<p>Elements of living systems that have <u>symbolic meaning</u>; Elements of living systems that have <u>sacred or religious meaning</u>; Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		<p>Other biotic characteristics that have a non-use value</p>	<p>Characteristics or features of living systems that have an <u>existence value</u>; Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

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

and

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



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Species Distribution Model

Projection of environmental suitability for *Misgurnus anguillicaudatus* establishment in Europe

Björn Beckmann, Hugo Verreycken and Dan Chapman

08 October 2020

Aim

To project the suitability for potential establishment of *Misgurnus anguillicaudatus* in Europe, under current and predicted future climatic conditions.

Data for modelling

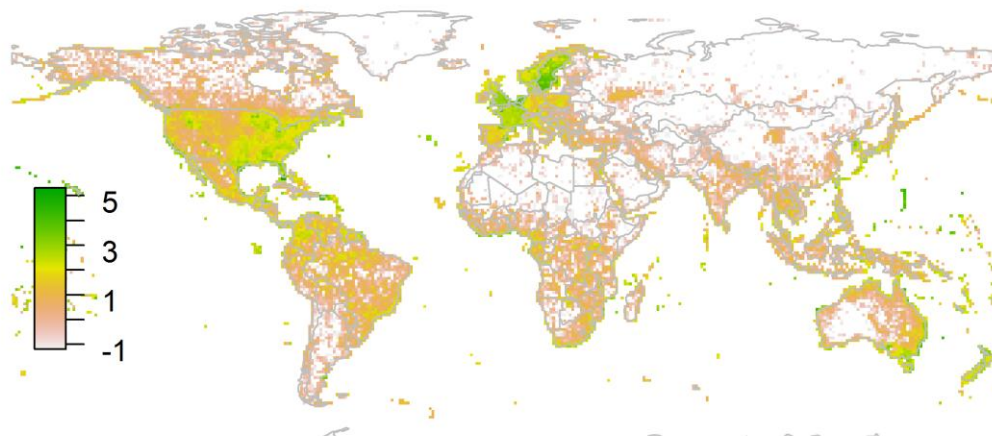
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (8448 records), the Atlas of Living Australia (979 records), the Integrated Digitized Biocollections (iDigBio) (219 records), iNaturalist (181 records), the Biodiversity Information Serving Our Nation database (BISON) (109 records), the Berkeley Ecoinformatics Engine database (1 records), and 18 additional records from the risk assessment team which were not included in the above and taken from individual publications. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 494 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Actinopterygii records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Misgurnus anguillicaudatus* and used in the modelling, showing native and invaded distributions. (b) The recording density of Actinopterygii on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Misgurnus anguillicaudatus*, the following climate variables were used in the modelling:

- Temperature seasonality (Bio4)
- Maximum temperature of the warmest month (Bio5)
- Minimum temperature of the coldest month (Bio6)
- Annual precipitation (Bio12)
- Precipitation seasonality (Bio15)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was $\ln+1$ transformed for the modelling to improve normality.

Species distribution model

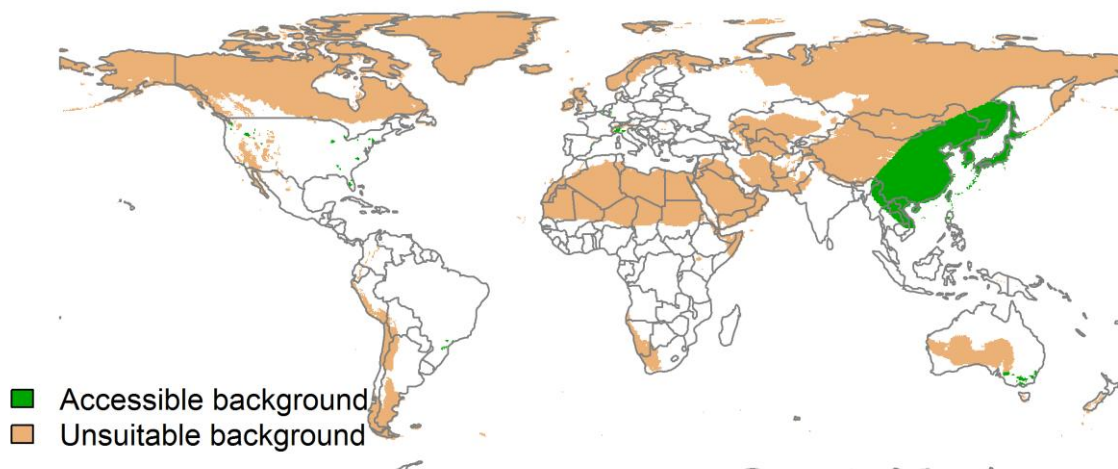
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental 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 (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Misgurnus anguillicaudatus* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the 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 so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Misgurnus anguillicaudatus* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -23°C
 - Maximum temperature of the warmest month (Bio5) < 19°C
 - Annual precipitation (Bio12) < 250mm

Altogether, only 1% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (494), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Misgurnus anguillicaudatus*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



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, five statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Random forest (RF)
- Maxent

Since the total background sample was 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 the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

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, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCDist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe 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.

Results

The ensemble model suggested that suitability for *Misgurnus anguillicaudatus* was most strongly determined by Human influence index (HII), accounting for 34.3% of variation explained, followed by Maximum temperature of the warmest month (Bio5) (27.4%), Annual precipitation (Bio12) (23.7%), Temperature seasonality (Bio4) (7.5%), Minimum temperature of the coldest month (Bio6) (6.9%) and Precipitation seasonality (Bio15) (0.1%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)					
					Human influence index (HII)	Maximum temperature of the warmest month (Bio5)	Annual precipitation (Bio12)	Temperature seasonality (Bio4)	Minimum temperature of the coldest month (Bio6)	Precipitation seasonality (Bio15)
GLM	0.962	0.632	0.892	yes	31	32	20	14	2	0
GAM	0.963	0.630	0.894	yes	30	24	27	7	13	0
GBM	0.962	0.636	0.890	yes	42	26	24	1	6	0
RF	0.944	0.607	0.883	no	24	22	35	8	7	5
Maxent	0.957	0.634	0.891	no	18	22	27	5	24	4
Ensemble	0.964	0.633	0.893		34	27	24	8	7	0

Figure 3. Partial response plots from the fitted models. 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.

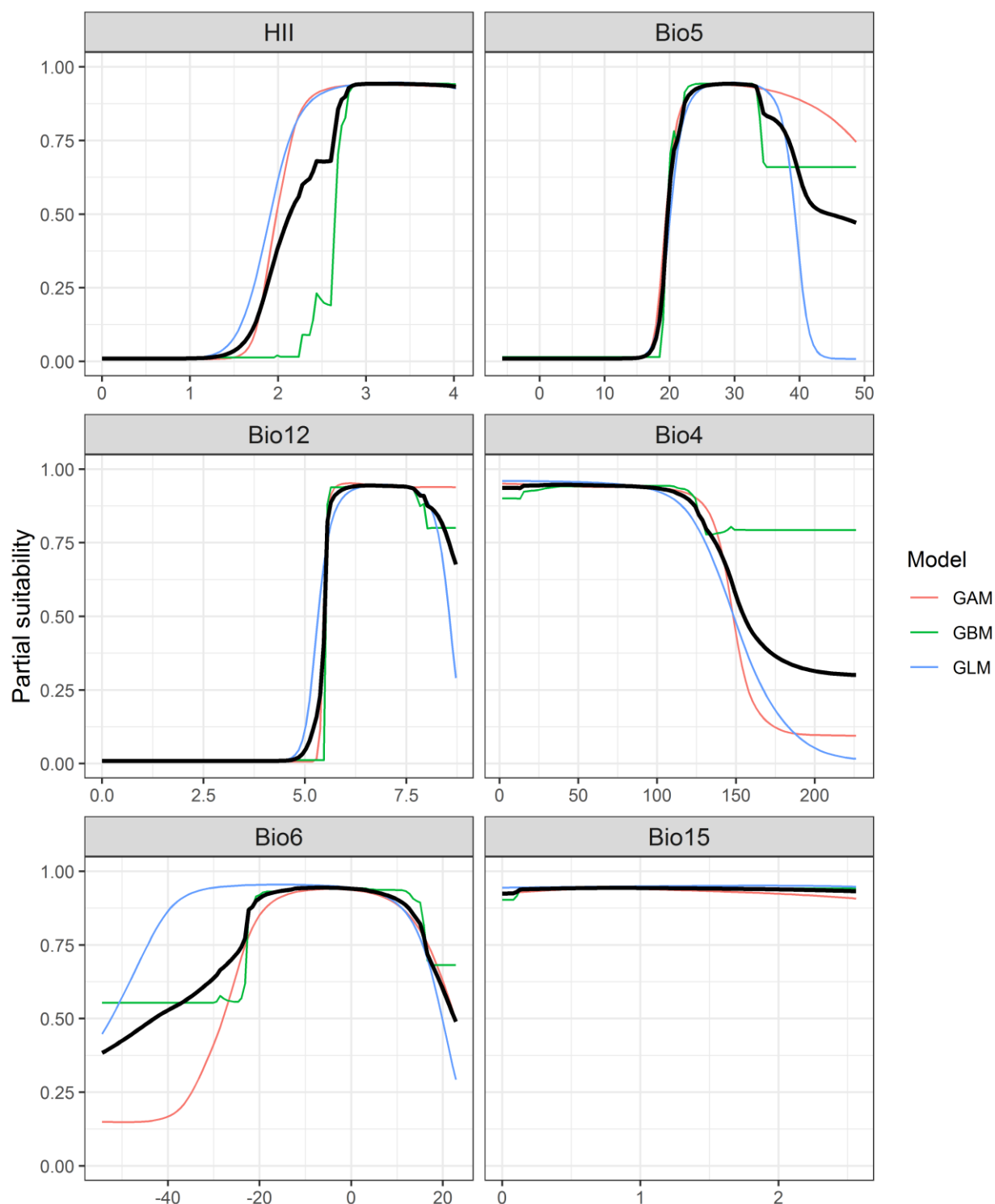


Figure 4. (a) Projected global suitability for *Misgurnus anguillicaudatus* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.43 may be suitable for the species. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

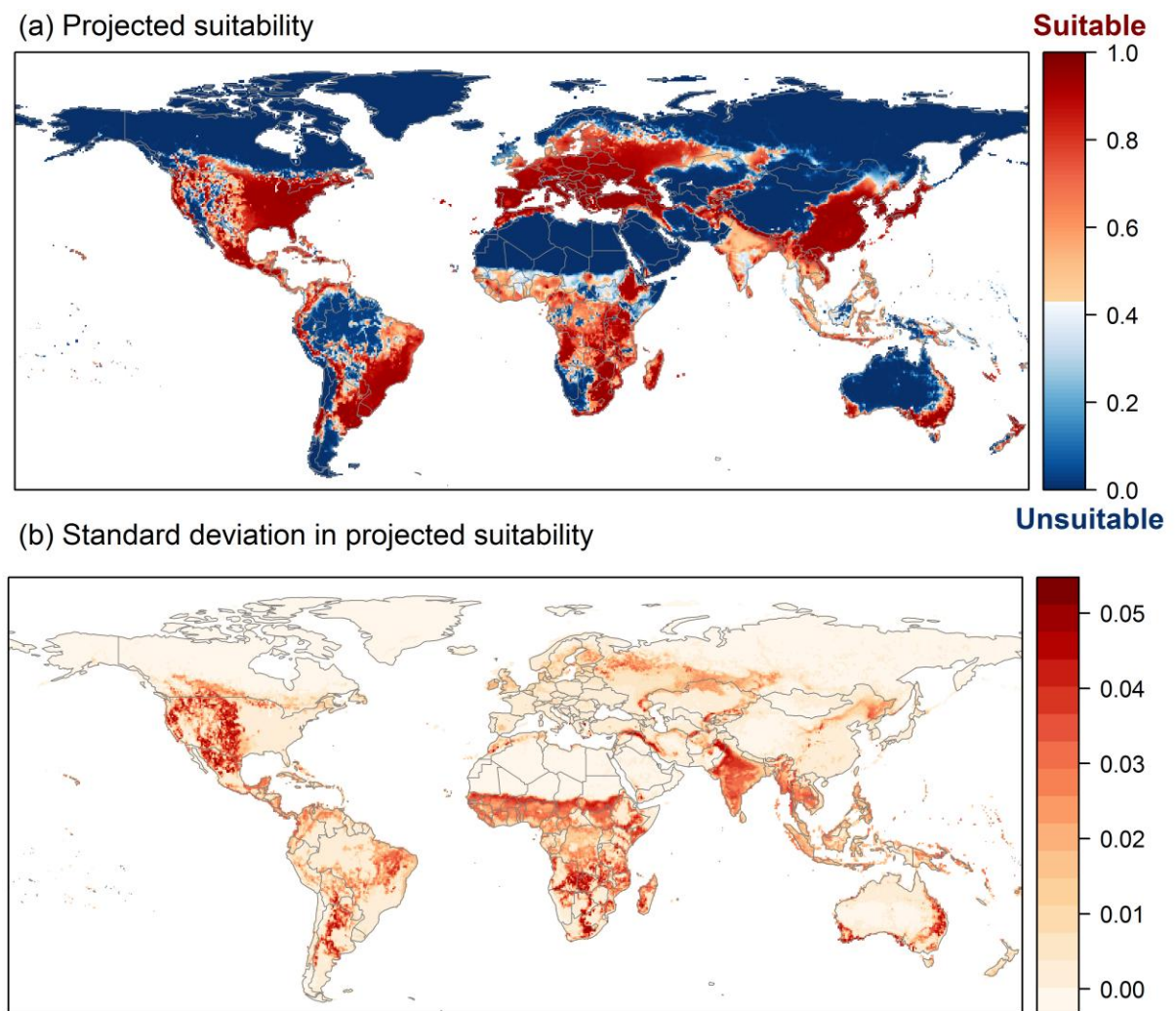


Figure 5. (a) Projected current suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

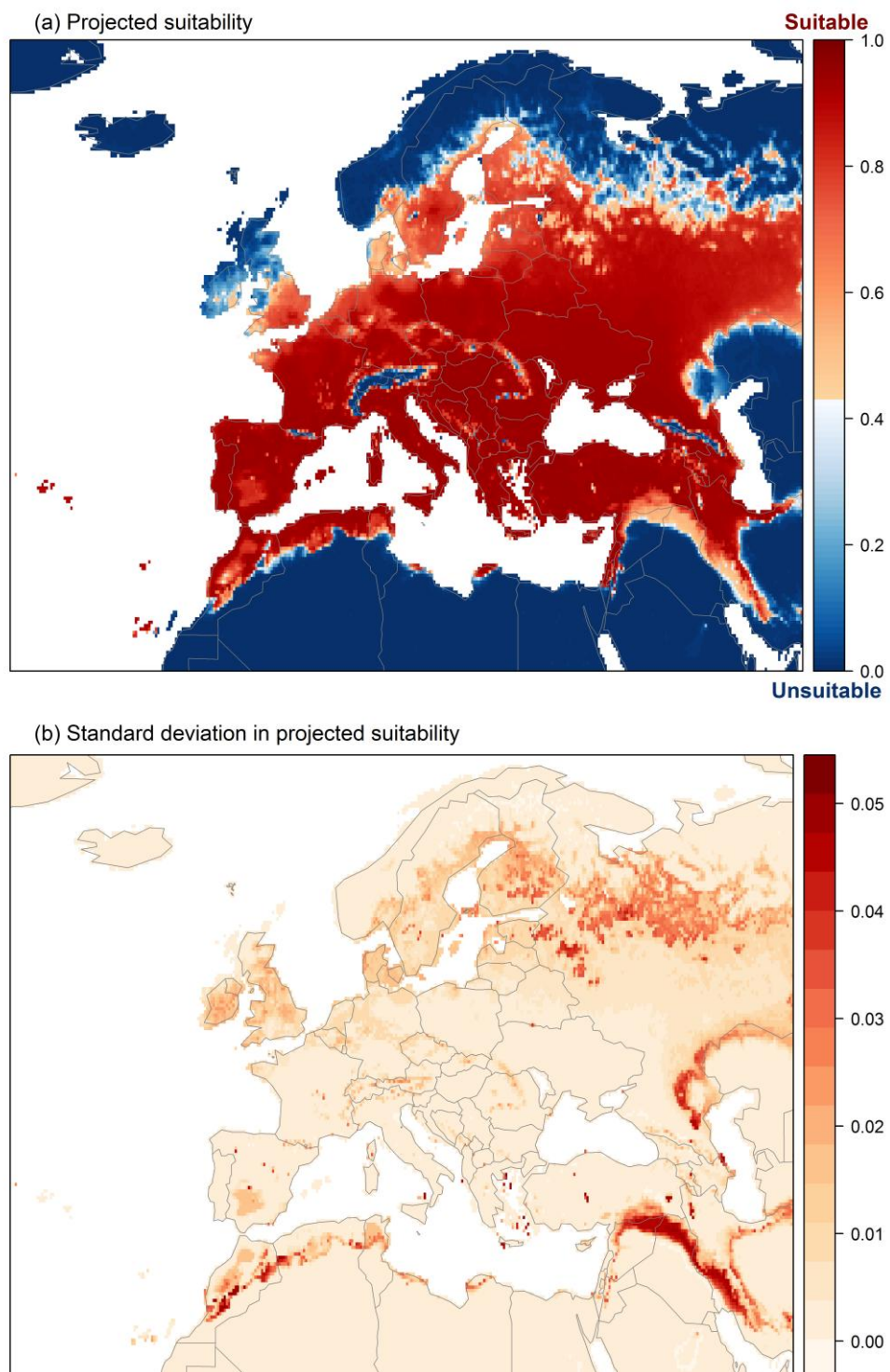


Figure 6. The most strongly limiting factors for *Misgurnus anguillicaudatus* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

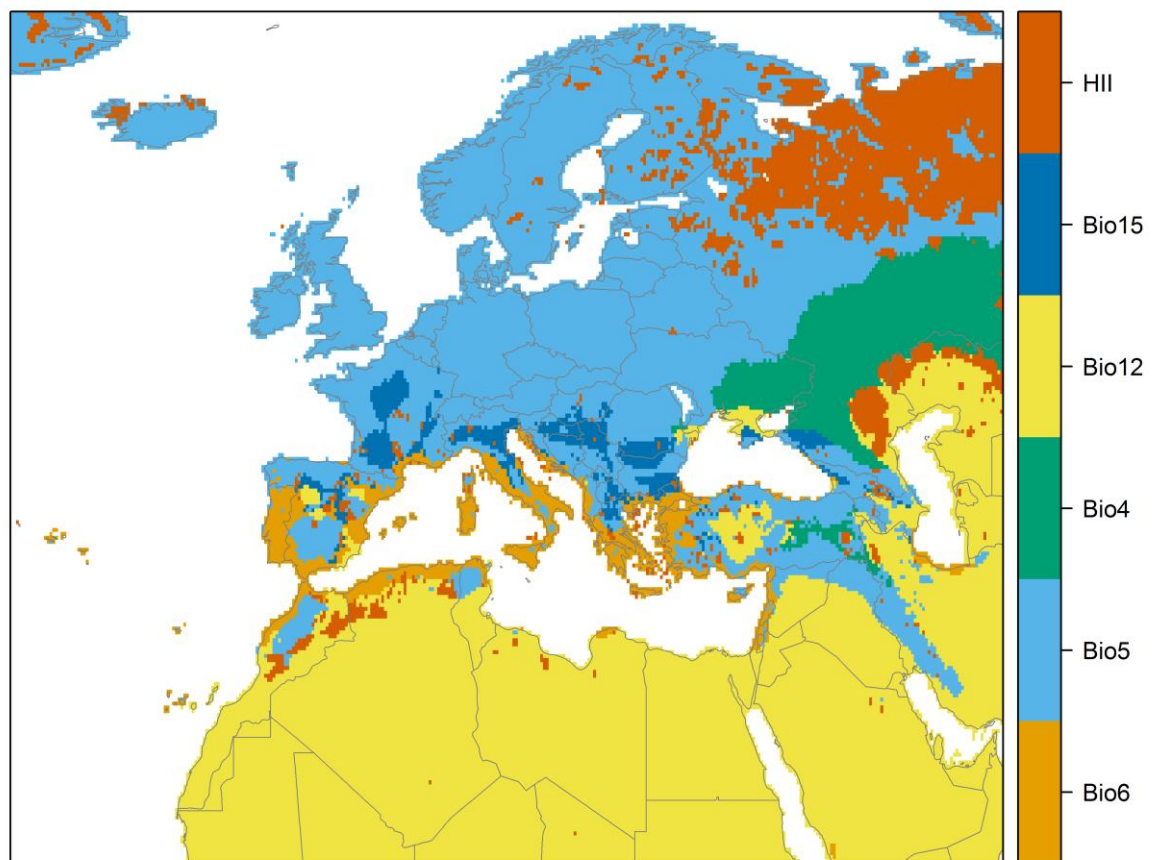


Figure 7. (a) Projected suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

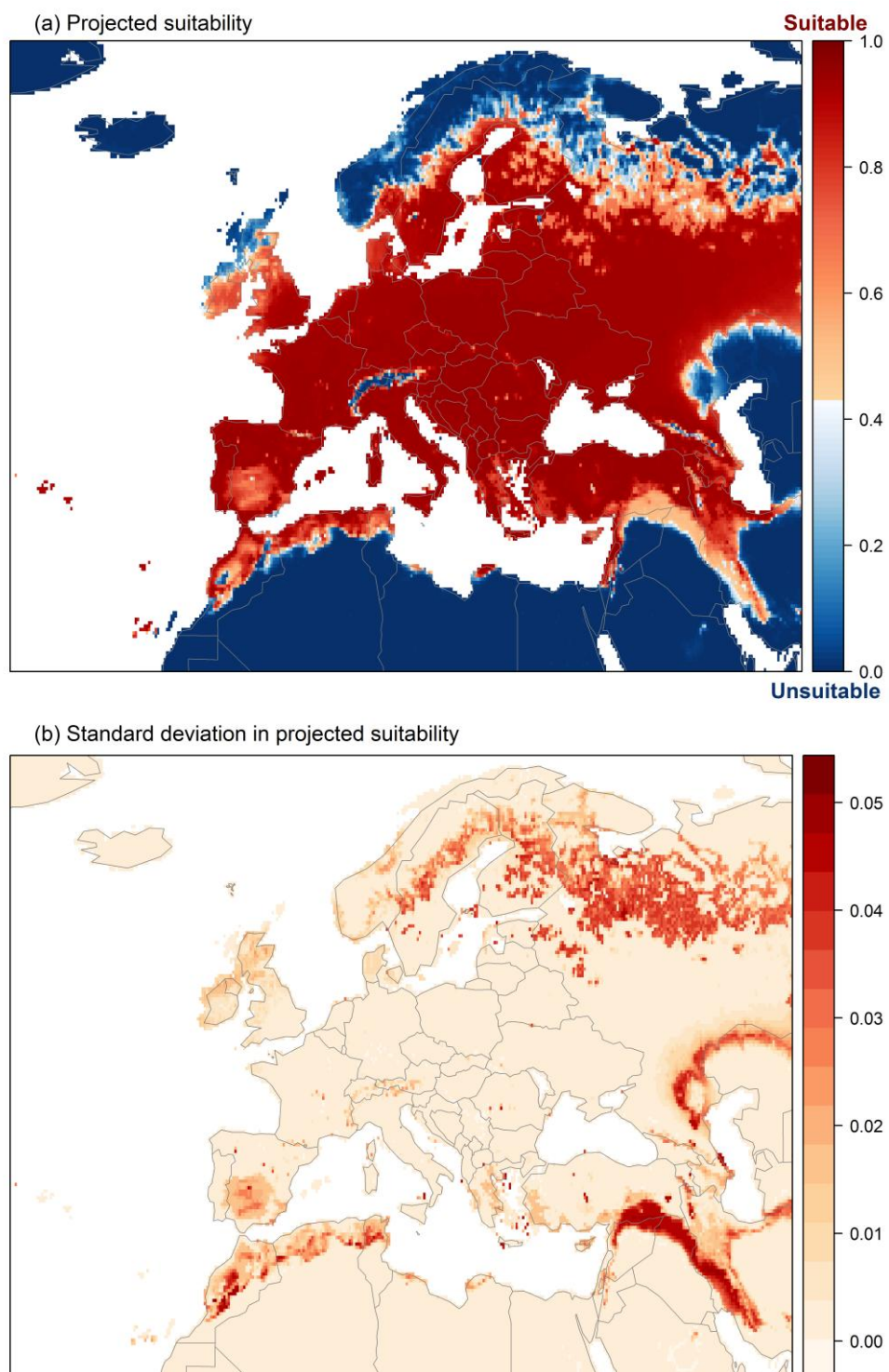


Figure 8. (a) Projected suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

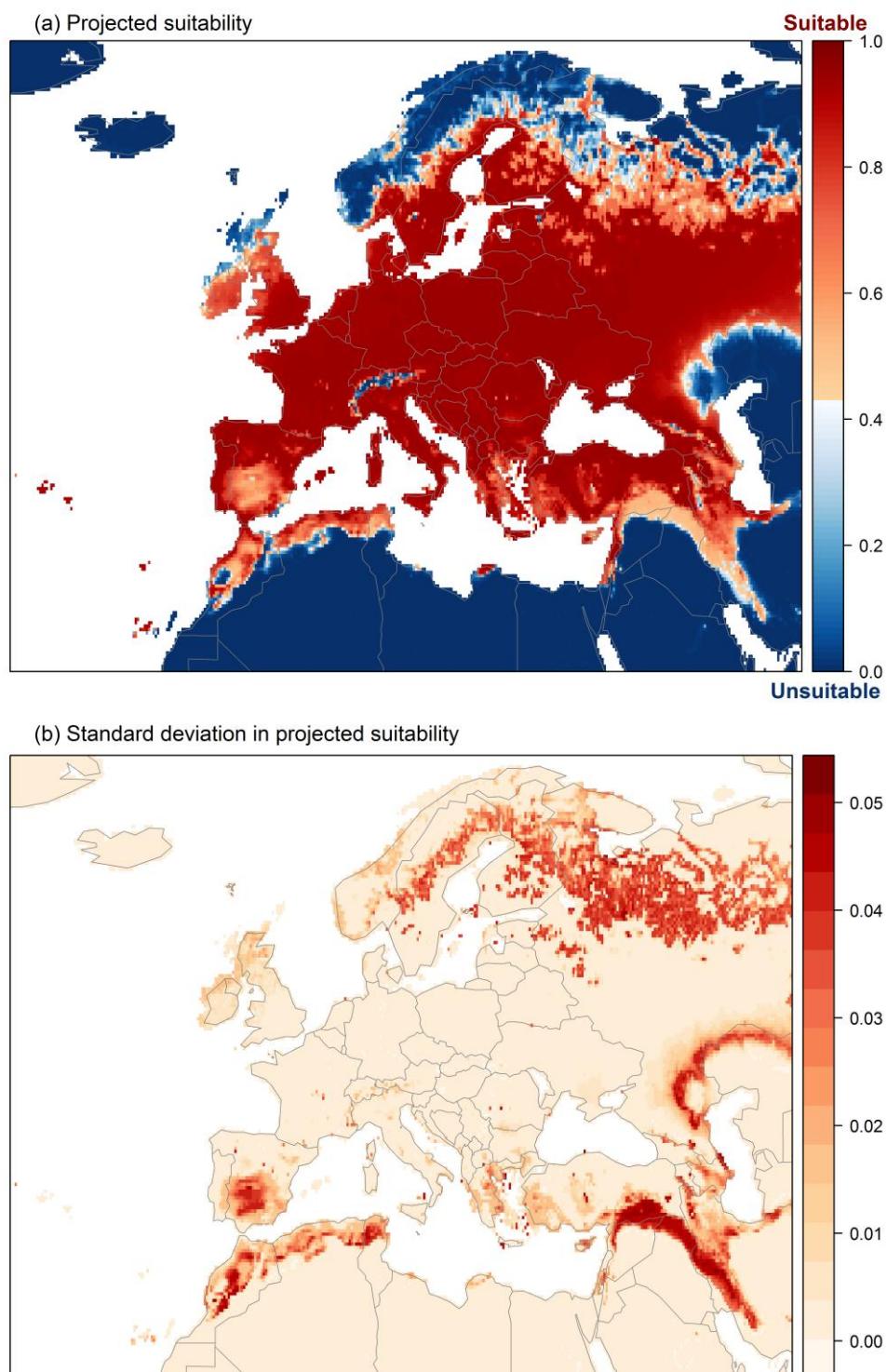


Figure 9. Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

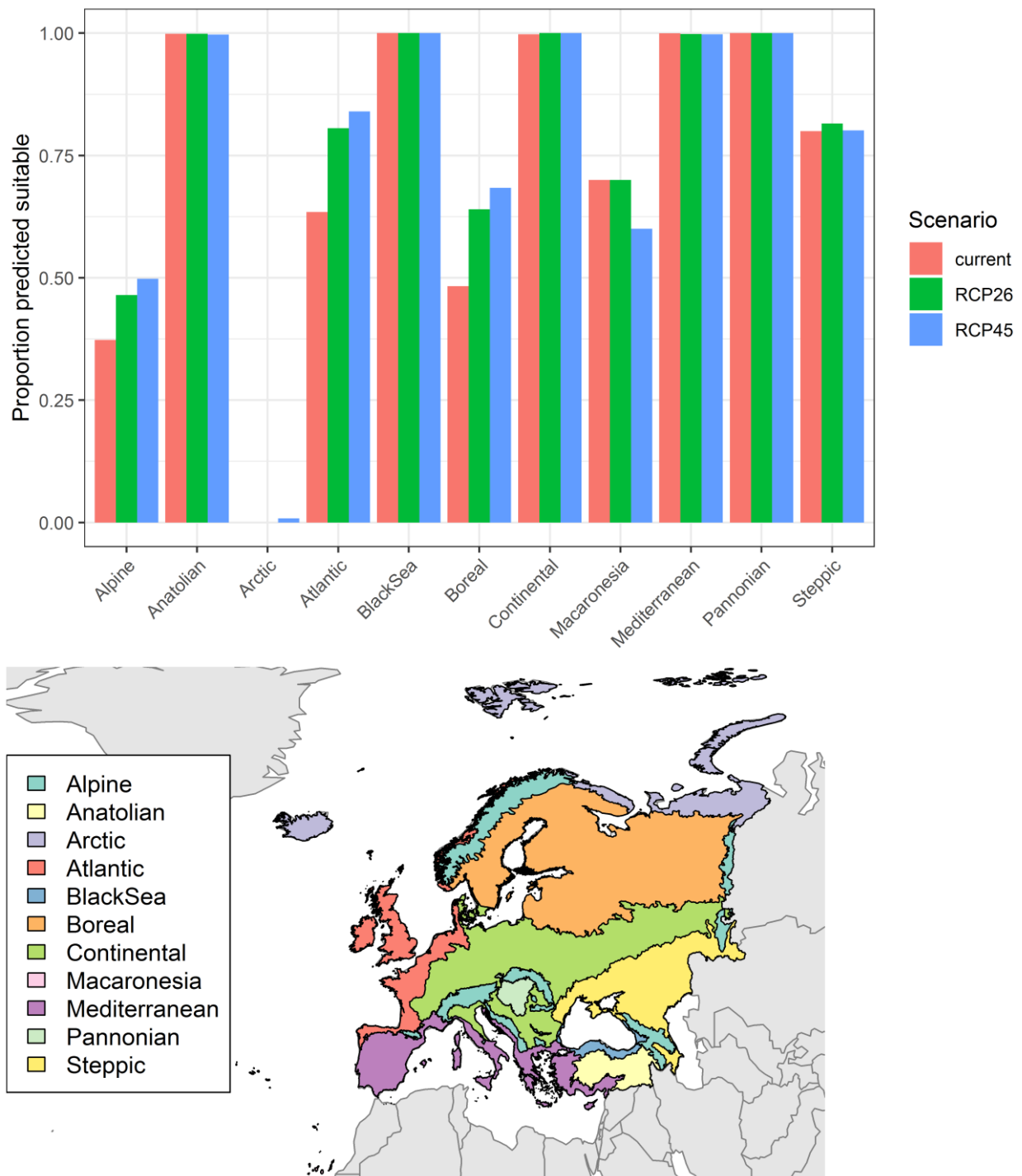


Table 2. Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

Region	current climate	RCP26	RCP45
Alpine	0.37	0.46	0.50
Anatolian	1.00	1.00	1.00
Arctic	0.00	0.00	0.01
Atlantic	0.63	0.81	0.84
BlackSea	1.00	1.00	1.00
Boreal	0.48	0.64	0.68
Continental	1.00	1.00	1.00
Macaronesia	0.70	0.70	0.60
Mediterranean	1.00	1.00	1.00
Pannonian	1.00	1.00	1.00
Steppic	0.80	0.82	0.80

Figure 10. Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

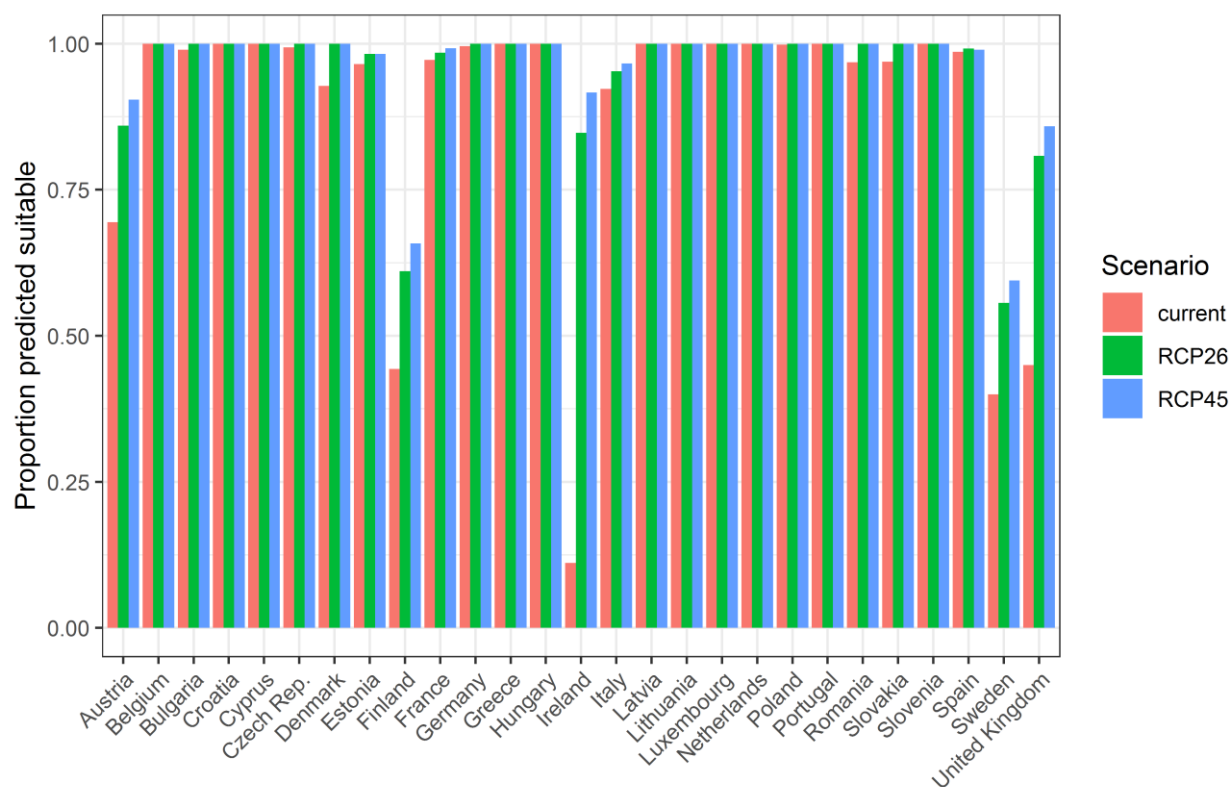


Table 3. Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

Region	current climate	RCP26	RCP45
Austria	0.69	0.86	0.90
Belgium	1.00	1.00	1.00
Bulgaria	0.99	1.00	1.00
Croatia	1.00	1.00	1.00
Cyprus	1.00	1.00	1.00
Czech Rep.	0.99	1.00	1.00
Denmark	0.93	1.00	1.00
Estonia	0.96	0.98	0.98
Finland	0.44	0.61	0.66
France	0.97	0.98	0.99
Germany	1.00	1.00	1.00
Greece	1.00	1.00	1.00
Hungary	1.00	1.00	1.00
Ireland	0.11	0.85	0.92
Italy	0.92	0.95	0.97
Latvia	1.00	1.00	1.00
Lithuania	1.00	1.00	1.00
Luxembourg	1.00	1.00	1.00
Netherlands	1.00	1.00	1.00
Poland	1.00	1.00	1.00
Portugal	1.00	1.00	1.00
Romania	0.97	1.00	1.00
Slovakia	0.97	1.00	1.00
Slovenia	1.00	1.00	1.00
Spain	0.99	0.99	0.99
Sweden	0.40	0.56	0.59
United Kingdom	0.45	0.81	0.86

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background 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 provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover were not included in the model.

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