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Review Non-native freshwater fauna in Portugal: A review



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HIGHLIGHTS

GRAPHICAL ABSTRACT

- We reviewed information on nonnative freshwater fauna in Portugal.
- Fish and mollusks are the taxonomic groups with more established species.
- Most species are native from other regions of Europe and North America.
- The Portuguese and EU legislation showed large discrepancies in its invasive species.
- We identified invaders for which legislation and actions are needed.



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ABSTRACT

We present the most updated list of non-native freshwater fauna established in Portugal, including the Azores and Madeira archipelagos. This list includes 67 species at national level but corresponds to 84 species records, of which 53 are in the mainland, 23 in the Azores and 8 in Madeira archipelagos. We also discuss the progression of the cumulative number of introductions since 1800 and identify the most probable vectors of introduction, main taxonomic groups and their regions of origin. Furthermore, we review the existing knowledge about ecological and economic impacts, invasion risk and potential distribution of invaders, under present and future climatic conditions, and the applied management actions, including the production of legislation. Along the 20th century the number of successful introductions increased at an approximate rate of two new species per decade until the beginning of 1970s. Since then, this rate increased to about 14 new species per decade. These introductions were mainly a result of fisheries, as contaminants or for ornamental purposes. Fish and mollusks are the taxonomic groups with more established species, representing more than half of the total. Most species (>70%) are native from other regions of Europe and North America. Studies about ecological or socioeconomic impacts are more common for fish, crustaceans and mollusks. Impacts for most amphibians, reptiles and mammals are not thoroughly studied. A few studies on the impacts and management actions of health-threatening mosquitoes are also available. The potential distribution in the Portuguese territory was modelled for 26 species. Only a minority of these models provides projections of distributions under scenarios of future climate change. A comparison of the Portuguese and EU legislation shows large discrepancies in the invasive species lists. Using the EU list

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and a ranking procedure for the national context, we identify freshwater species of high national concern for which actions are urgently needed.

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1. Introduction

The growing use of inland waters for recreation, food provisioning or navigation led to an increase in connectivity between aquatic systems and to the introduction of numerous non-native species. As a consequence, we now witness a growing homogenization of aquatic communities (Rahel, 2007; Villéger et al., 2011). Freshwater ecosystems present great declines in their biodiversity worldwide (Dudgeon et al., 2006; Millennium Ecosystem Assessment, 2005) and biological invasions are considered a major cause for those declines (Sala et al., 2000; Strayer and Dudgeon, 2010). The relative isolation of most freshwater ecosystems allowed the evolution of numerous endemisms and this is also the case in Portuguese freshwaters. The Pyrenean desman (Galemys pyrenaicus), fishes such as the saramugo (Anaecypris hispanica), or the unionid mussel (Unio tumidiformis) are iconic examples of endemic Iberian freshwater fauna occurring in Portugal, which are vulnerable to biological invasions (Barbosa et al., 2009; Cabral et al., 2005; Reis and Araujo, 2009). The Portuguese mainland shares most of its large river basins with Spain, and policies, laws and management actions are mostly implemented at a national level without full cooperation between the two countries. This may be problematic because the behaviour associated with the introduction of non-native freshwater fauna is distinct between these Iberian countries. Therefore, the adoption or voting of international conventions such as European Union (EU) regulations, needs to be framed within the context of each nation.

In Portugal, most studies about biological invasions in freshwater ecosystems have been directed to single species or specific groups (mainly fish, bivalves and crayfish). There is currently no full revision updating the list of non-native species and summarizing vectors of introduction, ecological and economic impacts and possible management actions to mitigate these impacts. This review summarizes the current knowledge about the history and introduction rates, main pathways, original locations, and ecological and socioeconomic impacts of nonnative freshwater fauna in Portugal (mainland, Azores and Madeira archipelagos). In addition, the invasion risk and potential distribution of these species under present and future climatic conditions, plus the management actions applied so far in Portugal are also discussed. This knowledge is vital for the design of an effective national invasive species monitoring plan and the future implementation of management actions and legislation.

2. Methods

Information on successful introduction records (i.e. non-native species that have established in the wild) for the Portuguese continental area (i.e. mainland), Madeira and the Azores archipelagos, from the 17th century to the present date, was compiled. We included species capable of living in freshwater at least during a part of its life-cycle and excluded cryptogenic, casual, or euryhaline species occurring only occasionally in freshwater. The following taxonomic groups of organisms were considered: "Mollusks", "Crustaceans", "Other Invertebrates", "Fish" and "Amphibians, Reptiles and Mammals". Other invertebrates included insects and non-arthropod species such as Cnidarians, Platyhelminthes, Nematodes and Annelids. Because of their great mobility and difficulty to distinguish accidental from established species, non-native aquatic birds were excluded from our analyses, except in the case of risk assessment of invasive species included in European Union legislation (see below for further details).

The native range of the species was divided into Europe, Africa, Asia, North America, South America and Oceania. Whenever a native distribution included more than one region (e.g. Europe and Asia), the closest region to Portugal was considered (e.g. Europe). The identification of the main pathway of introduction was primarily based on the published literature for Portugal or, when absent, on author's own knowledge. When information was not available from neither of these sources, we used the information available on the European Alien Species Information Network (EASIN) database (Katsanevakis et al., 2015). Nine EASIN categories were considered: "Fisheries", "Contaminant", "Stowaway", "Ornamental", "Biological Control and Research", "Aquaculture", "Animal Production", "Others" and "Unknown".

We reviewed the main ecological and economic impacts and distribution data for the above-mentioned fauna, as well as predictions from species distribution models (SDMs) for the current environmental conditions and under scenarios of future climates. National and international legislation was also reviewed, as well as management actions implemented so far. Freshwater species mentioned in the latest EU list of invasive species (EU Regulation 1143/2014 - EU, 2014) but not occurring in Portuguese inland waters were ranked in terms of likelihood of introduction or dispersal to the Portuguese mainland. The following criteria were applied in sequence, to produce a rank from 5 (already in national territory) to 1 (lowest likelihood): occurrence in the wild in Portugal (yes, no), captive populations in Portugal (e.g. aquarium trade or aquaculture - yes, no), occurrence in the wild in Spain (yes, no), distance to the Portuguese territory in km: 0, [0, 100], [100, 500] and >500. Species with wild populations in the mainland were automatically ranked as 5. The ones not in the wild but found in captivity were ranked as 4. A rank of 3 was attributed to species with wild populations in Spain at distances up to 100 km from the Portuguese border. Rank 2 was attributed to species with wild populations in Spain at distances from 100 to 500 km, and a rank of 1 to species at distances longer than 500 km from the Portuguese border.

3. Timeline and national statistics

100

80

60

40

20

0

1800 1820 1820 1860 1880 1920 1920 1920 1920 1980 1980 1980

Cumulative Number

In total, 84 first records of non-native species successfully introduced (i.e. established) were compiled (See Supplementary material I). This list includes 67 species at the national level, of which 53 are in mainland Portugal, 23 in the Azores and 8 in Madeira archipelagos. The number of species established in the mainland is slightly lower than the one provided by other authors for the Iberian Peninsula (73) but larger than the number provided for the neighboring Spanish region of Galicia (Cobo et al., 2010; García-Berthou et al., 2007). Since the numbers and the species differ, this suggests a possibility for future bidirectional cross-border dispersal.

The first introduction records are previous to 1800 and include carp (*Cyprinus carpio*) (1758) and goldfish (*Carassius auratus*) (c. 1792). The number of records increased considerably by the end of the 19th century due to a set of fish introductions in the Azores archipelago (Fig. 1). According to Goubier et al. (1983), these introductions were a private initiative of J.M. Raposo de Amaral, which introduced six nonnative species into the "Furnas" and "Sete Cidades" volcanic lakes.

During the 20th century the number of non-native species increased at an approximate rate of two species per decade until the early 1970s and then the rate markedly increased to about ten species per decade. After 1970 several well-known invaders were introduced in the mainland, namely the African clawed frog (*Xenopus laevis*), the red swamp crayfish (*Procambarus clarkii*) and the Asian clam (*Corbicula fluminea*). Along the turn to the 21st century the rate continued to increase and is now at 14 species per decade.

The taxonomic groups with higher numbers of non-native species introductions are fish and mollusks, respectively with 31 and 18 records. Crustaceans and other invertebrates have 10 and 16 records, respectively, while amphibians, reptiles and mammals together have 9 (Fig. 2). Most non-native species are native to North America or Europe, accounting for 72.2% of the introductions, with 31 and 29 records, respectively (Fig. 3). Asia follows next with 16 records (19.3%) while Africa, Oceania and South America together contributed with 8.4% of the records. This pattern is not unexpected, considering that most of the European trade occurs within Europe or between Europe and North America (García-Berthou et al., 2005).

4. Introduction pathways

The major pathways were contaminant species and intentional introductions for enhancing fisheries activities, with 32.1% and 31.3% of the records, respectively (Fig. 4). The introductions as ornamentals were the third most relevant pathway, with 20.2% of the records. Other identified pathways include stowaway (4.8%), aquaculture (2.4%), biological control (2.4%), animal production (1.2%) or other pathways (2.4%). Introduction pathway is unknown for only 3.6% of the records (three species). Despite the original pathway of introduction, many invaders, such as *P. clarkii* can explore several types of vectors for secondary spread. These vectors include accidental and intentional human transport but also natural vectors such as birds (Anastácio et al., 2013; Banha and Anastácio, 2015; Banha et al., 2014).

In the Iberian Peninsula, dams, channels and water transfers now affect the natural connectivity of a large part of the river basins. As an example, in mainland Portugal there are very few natural lakes but there are numerous dams, and the latter are generally more susceptible to invasions (Johnson et al., 2008). In fact, there is a predominance of nonnative species in dams in the Mediterranean region of the Iberian Peninsula (Clavero and Hermoso, 2011; Clavero et al., 2013) and in what concerns fish, this is largely due to intentional introductions by fishermen.

In Portugal, there are systematic official records of governmental fish introductions since the 19th century to promote recreational fisheries (Lourenço, 2004). Over 41 million juveniles were used for stocking purposes until 1996, of which over 35% consisted of non-native species: 22% rainbow trout, 8% carp and 5% European lake trout (*Salmo trutta lacustris*) (Lourenço, 2004). Until the beginning of the 2000s there were official introductions of non-native salmonids in the mainland Portuguese territory (Lourenço, 2004), and currently there are still official non-native salmonid introductions, mostly in the Azores and Madeira archipelagos.

Aquarium trade is considered an important pathway of introductions (Maceda-Veiga et al., 2016; Padilla and Williams, 2004) and in Portugal, this is the second most relevant for freshwater fish (Ribeiro et al., 2009). Although there is a trend for deceleration of freshwater vertebrate introductions in the Iberian Peninsula (Cobo et al., 2010),



2020

A. alburnus

A. melas P. leniusculi

P. clarki

G. holbrook

Time



Fig. 2. Number of first records of non-native freshwater fauna in the Portuguese mainland, Azores and Madeira archipelagos per taxonomic group of non-native species; N = 83 first records.



Fig. 3. Number of first records of non-native freshwater fauna in the Portuguese mainland, Azores and Madeira archipelagos, per area of origin; N = 82 first records.

we still witness a rise of the ornamental species in Portuguese basins (Ribeiro et al., 2009 and Supplementary material I).

Several aquarium fish species were successfully introduced in Portugal, for example, the goldfish (*C. auratus*), mummichog (*Fundulus heteroclitus*), pumpkinseed (*Lepomis gibbosus*) and the chameleon cichlid (*Australoheros facetus*). There were also unsuccessful introductions of other ornamental species such as tinfoil barb (*Barbonymus schwanenfeldii*), *Pangasius* sp. (Rebelo pers. observation) and *Piaractus* sp. (Ribeiro et al., 2009). Palma (2012) studied the import of nonnative pet species from 2003 to 2007 and concluded that fish were the most imported group, with >10 million specimens. Another study found that 259 non-native fish species are traded on pet shops in the Lisbon area, of which the most common were goldfish and guppy (*Poecilia reticulata*) (Mourão, 2012). These two common aquarium pets were both introduced in the Iberian Peninsula (García-Berthou et al., 2007; Ribeiro et al., 2008) but only goldfish is found in Portugal (Mourão, 2012).

Species from other taxonomic groups were also introduced by the aquarium trade. The red eared slider (*Trachemys scripta*), and at least three other freshwater turtles (*Graptemys pseudogeographica*, *Pseudemys concinna* and *Pseudemys nelsoni*) are commonly sold as pets (see Supplementary material I). Although the national law (D.R., 1999) has partially addressed the problem by forbidding imports of all three subspecies of *T. scripta* since 1999, some people still maintain and breed the species at home. In some pet shops the subspecies *Trachemys scripta troostii* is still sold due to its similarity to *Pseudemys* spp., for which importation is allowed. A total of 468,136 non-native freshwater turtles were imported from 2003 to 2007, mainly *Pseudemys concinna* and *Graptemys kohnii* (Palma, 2012).



Fig. 4. Number of first records of non-native freshwater fauna in the Portuguese mainland, Azores and Madeira archipelagos, per pathway of introduction; N = 83 first records. The EASIN database notation was used, except for "Unknown" which corresponds to "Not Evaluated/Assessed or No Data".

Another example of an aquarium species that it is also used in laboratories is the African clawed frog (*X. laevis*). This species was introduced in Portugal in at least one river basin near Lisbon (Ribeira da Lage), now occurring also in a nearby stream (Ribeira de Barcarena) (Rebelo et al., 2010a). Colonization of the later stream may have been due to natural dispersal or human mediated. Three pathways of introduction are possible, since 1) the species was commonly used for pregnancy tests in the past, 2) it was used in a scientific laboratory of the region which suffered a major flood, and 3) it is also kept as an aquarium pet (Sousa et al., 2018).

5. Ecological and economic impacts

Not all introductions become a problem and some of the invasive species can also have socio-economic or conservational benefits (Schlaepfer et al., 2011; Vitule et al., 2012). However, this section focused mainly on the negative ecological and economic impacts of the non-native species introduced in Portugal, presenting some examples from the main taxonomic groups.

5.1. Mollusks

Although mollusks comprise >20% of the non-native species of Portuguese inland waters, few studies quantified their ecological and economic impacts. One exception is the Asian clam (C. fluminea), subject to a great number of empirical and manipulative experiments in natural conditions, mainly in the Minho River where the species attains very high densities (Sousa et al., 2007; Sousa et al., 2005; Sousa et al., 2008b; Sousa et al., 2008c). In this river the C. fluminea high filtration rates have ecological impacts, affecting phytoplankton and higher trophic levels, as well as altering the amount of suspended particles, influencing light penetration and submerged vegetation (Sousa et al., 2009). The Asian clam is also responsible for modifications in the structure and diversity of fungi and bacteria (Novais et al., 2016), macroinvertebrate (Ilarri et al., 2015; Ilarri et al., 2012; Novais et al., 2015a) and epibenthic (Ilarri et al., 2014) communities, due to the presence of shells, bioturbation activities and enrichment of organic matter in the sediments. Some studies also showed how massive mortalities of this species may affect soil chemistry and fungi, bacteria and macroinvertebrate communities, including on adjacent terrestrial areas (Novais et al., 2017; Novais et al., 2015b).

Some species benefit from *C. fluminea* presence by increasing their density and biomass, but others, such as native bivalves, are negatively affected (Sousa et al., 2014). Regarding economic impacts, *C. fluminea* may be responsible for clogging and corrosion of plumbing in industrial facilities such as water treatment plants, power stations, and agriculture facilities such as irrigation pipes, among others (Rosa et al., 2011b). It is also responsible for negative impacts on fisheries due to the additional time needed to clean fishing gear, as well as negative effects on tourism due to the accumulation of shells in fluvial beaches (Sousa et al., 2008a; Sousa et al., 2008b).

The tadpole snail (*Physella acuta*) is another invasive mollusk with described ecological impacts. It affects rice production by consumption of rice seedlings (Vianna e Silva, 1969, 1975) and hosts several parasites affecting human health. Among the later, *Furcocercus cercaria* may cause cercarian dermatitis in humans, and this parasite was detected in the south of Portugal (Conchinha, 2016; Conchinha et al., 2015).

5.2. Crustaceans

A few non-native freshwater crustaceans occurring in Portugal (e.g. *P. clarkii, Pacifastacus leniusculus, Eriocheir sinensis* and *Crangonyx pseudogracilis*) had its ecological and economic impacts evaluated. *Procambarus clarkii* is a well-known rice production pest (Anastácio et al., 2005; Anastácio et al., 2000; Anastácio and Marques, 1997; Anastácio et al., 2005c), affecting young plants and seeds and promoting

water loss from rice fields. Additionally, its presence can attract high densities of predatory birds (e.g. white storks), which damage rice plants by trampling. Infestations at experimental densities of one crayfish m^{-2} decreased rice grain production by 41.6% (Anastácio et al., 2000) and in 2004 rice farmer associations estimated a loss of approximately 43€ per ha. However, the latter did not account for indirect losses due to, for example, levees reconstruction or water leakage from flooded fields. This represents more than one million euros of damage per year at the national scale. Studies addressing impacts of the red swamp crayfish also encompassed examples demonstrating negative effects on plants and algae (Anastácio et al., 2000; Barradas et al., 2006), aquatic macroinvertebrates (Banha and Anastácio, 2011; Correia and Anastácio, 2007; Correia et al., 2005), amphibians (Cruz and Rebelo, 2005; Cruz et al., 2006; Cruz et al., 2008; Nunes et al., 2010; Nunes et al., 2013), decomposition processes (Carvalho et al., 2016, 2018) and water quality (Anastácio et al., 2005b). Despite this, the species became an abundant food source for several native predators (Correia, 2001) and is also captured for commercial purposes, being mostly exported to processing units in southern Spain (Gutiérrez-Yurrita et al., 1999; Souty-Grosset et al., 2016).

The Chinese mitten crab (*E. sinensis*), which is present in the Tagus river since the 1980s, is also commercially captured mostly to supply demand from the local Asian community. This species is considered a nuisance due to damages to fishing gear and consumption of fish in fishing nets (Coelho, 2014) and it frequently contains microplastics (Wojcik-Fudalewska et al., 2016).

5.3. Other invertebrates

The Yellow Fever Mosquito (Aedes aegypti) is one of the main vectors of diseases such as dengue fever, yellow fever and zika (Musso et al., 2015). The species was present in mainland Portugal until 1956 and in 2005 was observed in Madeira island for the first time (Margarita et al., 2006). From late 2012 to early 2013 this mosquito was responsible for the first ever outbreak of dengue in Madeira, the first recorded in Europe since 1927, having infected >2000 people. Some concern exists that this new population in Madeira may act as a source for recolonization of mainland Portugal and other European countries (Almeida et al., 2007). This possibility is even more pronounced in the context of climate change, which is significantly increasing the extent of areas that are prone to the establishment of this mosquito in temperate regions (Capinha et al., 2014). A different species of concern, Aedes albopictus, which is also a competent vector of a number of arboviral diseases including dengue, was detected in the Portuguese mainland during 2017 (DGS, 2017).

5.4. Fish

Like other faunal groups, non-native fish in Portugal (and elsewhere) may have multiple-level impacts, including hybridization, disease transmission, disruption of biotic interactions and ecosystem wide effects (see reviews in Leunda, 2010; Ribeiro and Leunda, 2012). For example, the bleak (*Alburnus alburnus*) is a central European cyprinid that was recorded in Portugal in 2001 and is nowadays present in almost every basin (Latorre et al., 2018). The bleak hybridizes with the endemic cyprinids (*Squalius pyrenaicus* and *Squalius alburnoides*) (Almodóvar et al., 2012), being this phenomenon more common in the southern basins (Ribeiro pers. observation).

The European eel's (*Anguilla anguilla*) parasite [*Anguillicoloides* (\approx *Anguillicola*) *crassus*] is the single well known example of parasite transmission from the Japanese eel (*Anguilla japonica*) - a non-native fish used in aquaculture in Europe (Costa-Dias et al., 2010). Yet, only recently the parasites of non-native fishes started to be evaluated in Portugal (Kvach et al., 2017). Non-native fish can also disrupt host-affiliate relationships. For example, Douda et al. (2013) showed that non-native fishes in Portugal and Czech Republic are not suitable

hosts for the freshwater mussel *Anodonta anatina*, with potentially broad consequences for their population dynamics and conservation.

There is a lack of studies addressing resource competition (space and food) between native and non-native fishes in Portugal (Ribeiro and Leunda, 2012). However, recently, Kodde et al. (2016) showed, in laboratory conditions, that pumpkinseed sunfish (*L. gibbosus*) and chameleon cichlid (*A. facetus*) were faster and more efficient foragers than the native chub *S. pyrenaicus*.

Currently, there are five piscivorous fish introduced in Portugal (Ribeiro et al., 2009), but their diet was only described for largemouth bass (*Micropterus salmoides*) (e.g. Godinho et al., 1997). This study showed some predation towards fish, but the non-native crayfish *P. clarkii* constituted the staple prey item of largemouth bass (Godinho et al., 1997; Godinho and Ferreira, 1994). Also regarding predation, the mosquitofish (*Gambusia holbrooki*) has been described as a voracious predator of eggs and larvae of fish, amphibians, and invertebrates (Caiola and Sostoa, 2005; Vannini et al., 2018) but its benefit as a biological control agent remained uncertain (Mieiro et al., 2001). Additionally, this species is highly aggressive towards other fish species (Carmona-Catot et al., 2013; Magellan and García-Berthou, 2016).

Some of the non-native fishes, such as largemouth bass and common carp, are often preferred by recreational anglers in Portugal (Banha et al., 2017a) and may potentially generate economic benefits to some regions (Marta et al., 2001). Yet, economic damages caused by loss of ecosystems services (e.g. water quality degradation) may result in millions of euros as well (Walsh et al., 2016).

5.5. Amphibians, reptiles and mammals

The African clawed frog X. laevis is the single non-native amphibian that established invasive populations in mainland Portugal, being present in two small streams (Rebelo et al., 2010b). At least one species of flatworm (Protopolystoma xenopodis) is carried by these X. laevis individuals; however, this is a highly specialized species that was unable to parasitize native frogs (Rodrigues, 2014). Likewise, screenings for Batrachochytrium dendrobatidis, for which X. laevis is an asymptomatic vector to native amphibians (Solís et al., 2010), have been negative so far (Rebelo et al., 2016). The consumptive impacts of X. laevis in Portugal were assessed via stomach content analyses in both streams - Laje (Amaral and Rebelo, 2012) and Barcarena (Courant et al., 2017) indicating that the species consumes mainly benthic invertebrates. Predation over endangered vertebrates (fish and amphibians) occurs in both streams, but with a low frequency. Probably, the major impacts over the native communities occur through competition for invertebrate prey with native and endangered fish during late spring and summer, as water recedes and both fish and frogs become trapped in small pools.

Although no studies of non-native aquatic reptiles' impacts were performed in Portuguese territory some data exist for Spain, which has similar environmental and biological conditions (Mediterranean climate and shared native species). In Spain, the main negative impacts of *T. s. elegans* result from competition with native turtles (*Mauremys leprosa* and *Emys orbicularis*), especially for basking sites, due to the larger size and higher aggressiveness of *T. s. elegans*, and this situation may affect native turtles thermoregulation (Cadi and Joly, 2003). There are also reports of predation on invertebrates and fishes and their eggs and waterfowl nest disturbance (Martínez-Silvestre and Soler Massana, 2009; Perez-Santigosa et al., 2008). Amphibians may be especially vulnerable, since their tadpoles presented a naïve behavior to the novel predator, when compared to native turtles (Polo-Cavia et al., 2010).

Only one invasive mammal species is known in Portuguese freshwaters. Vidal Figueroa and Delibes (1987) registered the presence of the American mink (*Mustela vison*) for the first time in northwestern Portugal in 1985. According to the same authors, in the initial stages of invasion this species was a predator of marsh birds and their eggs, fish, small mammals (including the Iberian desman *G. pyrenaicus*; Rodrigues et al., 2015), amphibians and reptiles. However, more recent studies have shown that the main item of the specie's diet is the red swamp crayfish (Gonçalves, 2012). Therefore, it seems likely that *P. clarkii* facilitated the American mink's invasion and promoted the acceleration of the invasion front (Rodrigues et al., 2015).

6. Species distributions and invasion risk analysis

Species distribution models (SDMs) are fundamental to assess the risk of new invasions and to identify probable dispersal routes of ongoing invasions. According to our bibliographic review, 26 non-native freshwater faunal species, either already established in Portugal or under risk of establishment, were subjected to SDMs (Table 1). The most modelled taxonomic group was fishes (7 species), followed by crustaceans (5), mollusks (4 species and a multi-genus paper), reptiles (4), insects (3), amphibians (2) and mammals (1).

Projections for species that are not yet present (Table 1: yabbie, marmorkrebs, zebra mussel, apple snail, rotan and coypu) suggest that all have environmental conditions suitable for their establishment in the mainland. Additionally, models also show that most of the species that are established are not yet occupying all their potential distribution area. Exceptions are the red swamp crayfish and the Asian clam, both having already a very wide distribution in the mainland (Holdich et al., 2009; Rosa et al., 2011b). For apple snails (gen. *Pomacea*), there has been some taxonomic uncertainty. The species present in Spain, especially in the rice fields of the Ebro River, is believed to be *Pomacea maculata* (Salgado and Soriano, 2013), which has a large spreading potential across Spain and Portugal.

Until now, effects of climate change on species distribution were studied for four decapod crustaceans, three insects, one amphibian, one bivalve, one gastropod species and four gastropod genera (Table 1). For decapods, predictions under two future climate scenarios (B1, low greenhouse gas emissions and A2, high emissions) (IPCC, 2014), indicated a generalized loss of suitable area, mainly in the south and in the interior of the Portuguese mainland territory (Capinha et al., 2012). However, for the yellow fever mosquito the general trend is an increase in suitable area under all climate change scenarios. For the African clawed frog, model predictions also point to a loss of suitable area in the south, but this is compensated by an increase of

Table 1

Information obtained from the available distribution models for non-native freshwater fauna already established in Portugal or under risk of establishing in the mainland. Note that in the case of the apple snail (genus *Pomacea*), the species may be *P. maculata* and not *P. canaliculata*. When a species is only present in Madeira or Azores archipelagos, and not in mainland Portugal, this information is presented.

Species	Established?	Present conditions	Future climatic conditions	References		
		Unoccupied areas in mainland?	Extension of distribution in mainland?			
Yellow fever mosquito (Aedes aegypti)	Madeira	yes	Increase	(Capinha et al., 2014; Kraemer et al., 2015; Monaghan et al., 2018)		
Asian tiger mosquito (Aedes albopictus)	Mainland	Yes	Decrease	(Caminade et al., 2012; Cunze et al., 2016; Fischer et al., 2011; Kraemer et al., 2015)		
Water Boatman (Trichocorixa verticalis)	Mainland	Yes	Slight Decrease	(Guareschi et al., 2013)		
Red Swamp Crayfish (Procambarus clarkii)	Mainland, Azores	No	Decrease	(Capinha et al., 2012; Filipe et al., 2017b)		
Yabbie (Cherax destructor)	No	Yes	Decrease	(Capinha et al., 2012)		
Signal Crayfish (Pacifastacus leniusculus)	Mainland	Yes	Decrease	(Capinha et al., 2012; Filipe et al., 2017b)		
Marmorkrebs (Procambarus fallax)	No	Yes	-	(Feria and Faulkes, 2011)		
Chinese Mitten Crab (Eriocheir sinensis)	Mainland	Yes	Decrease	(Capinha et al., 2012)		
Asian Clam (Corbicula fluminea)	Mainland	No	Decrease	(Gama et al., 2016; Gama et al., 2017)		
Zebra Mussel (Dreissena polymorpha)	No	Yes	-	(Quinn et al., 2014)		
		No	-	(Gallardo et al., 2013)		
Apple Snail (Pomacea canaliculata)	No	Yes	_	(EFSA Panel on Plant Health, 2013)		
Wandering Snail (Radix balthica)	Azores and Madeira	No	No change	(Cordellier and Pfenninger, 2009)		
Several snail Genus	Mainland Azores or	Yes	No change except for	(Cordellier et al., 2012)		
(Galba, Gyraulus, Physa = Physella, Planorbarius, Radix)	Madeira		Physa (decrease)			
Eastern Mosquitofish (Gambusia holbrooki)	Mainland	Yes	-	(Murphy et al., 2015)		
Largemouth Bass (Micropterus salmoides)	Mainland, Azores	Yes	-	(Bae et al., 2018)		
Pumpkinseed (Lepomis gibbosus)	Mainland	Only one sub-basin was studied	-	(Ferreira et al., 2016)		
Gudgeon (Gobio lozanoi)	Mainland	Only one sub-basin was studied	-	(Ferreira et al., 2016)		
Common Carp (Cyprinus carpio)	Mainland, Azores	Yes	_	(DeVaney et al., 2009)		
		Yes	-	(Zambrano et al., 2006)		
Tench (Tinca tinca)	Mainland	Yes	-	(DeVaney et al., 2009)		
Rotan (Perccottus glenii)	No	Yes	-	(Reshetnikov and Ficetola, 2011)		
African Clawed Frog (Xenopus laevis)	Mainland	Yes	Increase	(Capinha and Pateiro-Lopez, 2014; Ihlow et al., 2016; Measey et al., 2012)		
Italian Crested Newt (Triturus carnifex)	Azores	Yes	-	(Wielstra et al., 2013)		
River Cooter (Pseudemys concinna)	Mainland	Yes	-	(Masin et al., 2014)		
Florida Red-bellied Cooter (<i>Pseudemys</i> nelsoni)	Mainland	Yes	-	(Masin et al., 2014)		
False Map Turtle (Graptemys pseudogeographica)	Mainland	Yes	-	(Masin et al., 2014)		
Red-eared Slider (Trachemys scripta)	Mainland	Yes	-	(Banha et al., 2017b; Rödder et al., 2009)		
Coypu (Myocastor coypu)	No	Yes	-	(Jarnevich et al., 2017)		

suitable area in the north (Capinha and Pateiro-Lopez, 2014). It is possible that this trend is shared by most of the freshwater invaders, due to the expected aridification of the South, but more studies would be necessary to corroborate this prediction (Seidel et al., 2008).

Mourão (2012) considered that a blacklist of fish invaders for Portugal should include the white cloud mountain minnow (Tanichthys albonubes), black molly (Poecilia sphenops), zebrafish (Danio rerio), southern platyfish (Xiphophorus maculatus), peppered corydoras (Corydoras paleatus) and green swordtail (Xiphophorus helleri). Another study added suckermouth catfish (Hypostomus plecostomus) to this list (Range, 2013). In 2013, another risk analysis concerning fish invasions in the Iberian Peninsula identified 24 species posing a high risk (Almeida et al., 2013). Meanwhile, five of these were detected in Portugal, namely channel catfish (Ictalurus punctatus) (Banha et al., 2017c), European perch (Perca fluviatilis) (Banha et al., 2015), Wels catfish (Silurus glanis) (Gkenas et al., 2015), common roach (Rutilus rutilus) (Ribeiro and Veríssimo, 2014) and Prussian carp (Carassius gibelio) (Ribeiro et al., 2015). Additionally, some of the species with a high invasion risk are already established in Spain, being almost inevitable the spread to Portuguese territory of the topmouth gudgeon (Pseudorasbora parva), pond loach (Misgurnus anguillicaudatus) and the rudd (Scardinius erythrophthalmus) (Almeida et al., 2013).

7. Management actions

As in other countries, in Portugal some of the most cost-effective means to deal with aquatic invasions are related to preventing the introduction and spread of species or detecting the invasions at a very early stage. Below, we discuss some of these management actions.

7.1. Prevention and early detection

Some Portuguese institutions (Parque Biológico de Gaia and RIAS-Aldeia) have been developing public awareness programs for decades, working with schools and offering an alternative to the release of pet turtles in the wild, by receiving unwanted pets. Similarly, in a local environmental project (Paeloris, https://www.cm-mira.pt/node/380) mainly devoted to the conservation of freshwater mussels, audiovisual materials were produced for public awareness regarding the possible effects of the Asian clam (*C. fluminea*) on those species, but also addressing other aquatic invaders.

Yet, these actions seem to have limited effects and a higher involvement of general society in invasive species awareness campaigns and actions is fundamental for preventing invasions and for early detection. In this context, social media have been demonstrated as a powerful source of information for the early detection of two fish introductions (*lctalurus punctatus* and *S. glanis*) (Banha et al., 2017c; Gago et al., 2016). Additionally, under the FRISK project (www.facebook.com/ FRISKPROJECT) public outreach directed to recreational fishermen aims to both increase environmental awareness and allow direct contact with fishermen records of non-natives.

A good example of prevention and early detection concerns the apple snails (genus *Pomacea*), which invaded Spanish territory but have so far not been detected in Portugal. Its potential effects on rice production can be serious and therefore, since 2016, there is a governmental contingency plan defining actions and procedures (DGAV, 2016). Apple snails are popular among aquarists but the sale of *Pomacea* species is now forbidden. Since 2013 the National phytosanitary authority established an annual *Pomacea* prospection program to be implemented throughout the country. It is not only directed to rice fields but also several other freshwater systems, including for example lakes in urban gardens. In the same vein, the import of aquatic plants depends on a phytosanitary certificate or a phytosanitary passport.

The possible imminent invasion by zebra mussel (*Dreissena polymorpha*), which is already established in several Spanish basins,

and its predictable high ecological and economic effects, led to proactive monitoring by entities of the private sector related with water use and management. Hydropower and water management companies are currently implementing detection programs, mainly directed to the larvae (Banha et al., 2018). These companies have also promoted awareness using outdoor panels. In addition to the ongoing attempts for early detection of several freshwater invaders, there are very promising new techniques such as environmental DNA which are already being tested in Portugal (Filipe et al., 2017a; Filipe et al., in press).

7.2. Control and containment

The unique successful eradication of an invasive faunal species in Portuguese freshwaters that we are aware deals with the Yellow Fever Mosquito. This species was present in mainland Portugal until 1956 (Almeida et al., 2007; Holstein, 1967) but it may have been introduced around the Mediterranean area as early as 1000 BCE (Manguin and Boëte, 2011). The possible disappearance of this species, which occurred at about the same time as in other Mediterranean countries (Holstein, 1967), was a likely result of widespread improvement of sanitary conditions and the application of Dichloro-diphenyl-trichloroethane (DDT) (Fontenille et al., 2007).

Despite a successful eradication in the mainland, *A. aegypti* recently invaded the Madeira island (see above). The consequent Dengue fever outbreak led to the preparation of an action plan to control and contain the problem (SRAS, 2013). Currently, the local health authorities have implemented a surveillance system based on the monitoring of the mosquito densities and of any suspected case of disease. Adult traps (BG-Traps) and egg traps (Ovitraps) are distributed throughout the island and verified daily or weekly, respectively (Silva et al., 2015). There is also a web tool for voluntary report of the mosquito occurrence in which it is possible to visualize the current intensity of the invasion throughout the island (http://iasaude.sras.gov-madeira.pt/naomosquito/). Actions for active control include intervention on potential nursery areas and education of the population to reduce the number of microhabitats available for mosquito reproduction.

In addition to the control campaigns devoted to yellow fever mosquito, the Asian clam C. fluminea, due to its economic impacts, has been subjected to some research directed to its control and/or containment. Although some monitoring and preventive procedures have been proposed (Gabriel et al., 2013; Rosa et al., 2011a; Rosa et al., 2011b), including in industrial contexts (Gabriel, 2011), there is no continuous monitoring program by governmental authorities or private entities (Gabriel et al., 2014). Regular cleaning actions are taken, e.g. in some irrigation systems, to prevent clogging by Asian clams and this may sum up to 2% of its operational costs (Rosa et al., 2011b). Gabriel (2011) while testing chemical control methods, demonstrated that niclosamide and dimethoate are toxic to the Asian clam, suggesting the possibility of its use in control programs. Results also showed that the combined effect of the two previous compounds is antagonistic, not recommending the use of a mixture for Asian clam's control in industrial environments. A later study by Rosa et al. (2015) indicated that hypoxia significantly increases the sensitivity of the Asian clam to dimethoate, ammonium nitrate, niclosamide, potassium chloride and polyDADMAC.

Despite the high impacts caused by the red swamp crayfish *P. clarkii* on rice production and its inclusion in the European Union's list of invasive species of concern, so far, no official program of control has been implemented in Portugal. However, due to its commercial exploitation, large quantities are harvested every year, for example in the Tagus river basin (Rodrigo et al., 2000). It was also common practice among farmers to use xenobiotic chemicals to control this pest. In the early 1990s the invasion made the headlines of the national media due to its impacts, visibility and due to the use of Dimethoate, Endosulfan and Parathion, with massive environmental consequences (Marques et al., 1992). Several unofficial initiatives or scientific projects were also carried on trying to mitigate the invasion, but none was successfully implemented.

Among these are the test of an oily emulsion to control the species (Ganhão et al., 1991), the development of a non-ionic surfactant to decrease the activity of the species during early stages of rice development (Anastácio et al., 2000) and the planning of integrated production systems with rice and crayfish (Anastácio et al., 1999a; Anastácio et al., 1995).

Regarding the control and/or containment of non-native fishes, the National Institute for Nature and Forest Conservation (ICNF) has been removing specimens in a stretch of the Vascão river in the Lower Guadiana Basin since 2007 (Cardoso, 2014; Cardoso and Carrapato, 2011). Currently, these actions are ongoing under a LIFE-Project, aiming to control the populations of the non-native fish largemouth bass, pumpkinseed sunfish and chameleon cichlid with volunteers, as part of a communication strategy to raise public awareness. Along the first eight years (2007-2014) of actions, a total of 14,200 non-native fish was removed, mainly sunfish and largemouth bass (Cardoso, 2014). The efforts done showed a continuous decrease of the proportion of non-native fish in their catches from 90% to 70%, being more efficient in reducing the populations of both centrarchids (largemouth bass and sunfish) (Cardoso, 2014). This continuous and ongoing effort to control non-native fish populations serves as a pilot study, and represents a very low cost to the agencies (i.e. 500 euros per year) (Cardoso, 2014). Additionally, in the Natural Park of the Guadiana Valley, two small irrigation reservoirs have been equipped with a fish barrier (steel structure, fence-like, see Rischbieter, 2000) in their outlet that prevents non-native fish to spread downstream. However, there is no current monitoring of the effectiveness of this equipment. No rotenone treatments were applied to control or locally eradicate nonnative fish in Portugal, despite their extensive use in several European countries (Morcillo et al., 2017). Considering the current rates of fish introduction and potential spread of EU List species from Spain, such as topmouth gudgeon, rotenone may be a management option.

The African clawed frog provides a good example of an eradication attempt directed to an invader. Since 2010, streams of the Lisbon area, suspected or at risk of invasion, are being monitored and removal is ongoing (Rebelo et al., 2010b). This process so far succeeded in containing the spread and significantly reduced frog abundance on both streams, although total eradication has not yet been accomplished. Adults were captured using baited funnel traps and electrofishing but eggs and tadpoles were also detected and actively removed (Rebelo et al., 2016).

Regarding the control and/or containment of aquatic invasive reptiles, one program was funded by the European commission, the Life Trachemys 09NAT/ES/00259. This involved three Portuguese and two Spanish entities from 2011 to 2013 and was focused on the conservation of the two native pond turtles, M. leprosa and E. orbicularis in the regions of Valencia, in south Spain and Algarve, in the south of Portugal. In Portugal this program acted in four costal lagoons of high conservation value included in the Natural Park of Ria Formosa, where these two native species were present, as well as an invasive population of T. scripta (Teixeira et al., 2013). The plan involved 3 people and some volunteers in 2011 and 2012, using traps (both floating and submerged), handcapture and dipnets. A total of 214 non-native turtles were captured during this period, 52% with traps, 32% by hand-capture during female terrestrial movements to nest and 16% by dipnet removal from public garden ponds. Most specimens were T. s. elegans (n = 176; 82%) followed by *T.s. scripta* (n = 20; 9%) and a few hybrids (n = 4; 2%) of *T.s.elegans* × *T.s.scripta*. Some individuals from other species were also found, such as *P. concinna* (n = 7; 3%), *G. pseudogeographica* (n = 6;3%) and *P. nelsoni* (n = 1; <1%). The majority were adults, however, 18 recently hatched T.s. elegans were captured in S. Lourenço lagoon, confirming that the regional environmental conditions are suitable for reproduction. During the program, the terrestrial surroundings of the intervened waterbodies were scanned for nests and this action allowed the removal of 112 eggs from 9 nests (LIFE-Trachemys, 2012).

8. Legislation

There is National and European legislation applicable to invasive species. National law specifies numerous restrictions and directives related to invasive species (D.R., 1999). As an example, any store selling ornamental plants or pets must hang on a visible location a copy of an official document (a leaflet) with information regarding dangers of invasive species, the legal restrictions related to them and the associated fines for breaking the law. In the scope of this legislation, several important freshwater non-native invasive species are specifically listed in two different annexes. Annex I contain a list of non-native fauna in each river basin, while annex III lists species with known ecological risk. The freshwater fauna in Annex I includes two mollusks, three crustaceans and 13 fish, which largely underestimates the current number of species in Portuguese freshwater ecosystems. In what concerns annex III (see Table 2), the list of freshwater fauna includes two mollusks, not yet occurring in Portuguese territory, and 16 species of fish of ecological concern. Of these fish, five already occur in Portugal. Four freshwater turtles and one amphibian were included in the same annex. However, only one turtle species has been detected in the wild. One bird is also in the annex as well as six mammals closely associated with water. Only the bird (Oxyura jamaicensis) and one mammal (Mustela vison) are currently found in Portugal.

Although recently disputed (Carboneras et al., 2018), there is EU Legislation and a list of invasive species of concern that must be adopted by the National authorities (European Commission, 2013, 2016). The invasive species in this list must be subjected to management measures to

Table 2

Freshwater species in the Annex III of the Portuguese legislation about exotic species, i.e. species with known ecological risk (D.R., 1999).

Taxonomic group	Established in Portugal?
Mollusks	
Dreissena polymorpha	No
Dreissena bugensis	No
r'.l.	
FISII Doreg fluvigtilic	Voc
Lenomic cyanallys	No
Lepomis cyunenus	NO
Lepoints gibbosus	No
Oreochromis piloticus	No
Oreochromis Intolicus	No
Tilania zilli	No
Tilania melanonleura	No
Stizostedion vitreum [Sander vitreum]	No
Stizostedion lucionerca [Sander lucionerca]	Ves
Gymnocenhalus cernuus	No
Hynonhthalmickthys molitrix	No
Osmerus mordax	No
Misgurnus anguillicaudatus	No
Gambusia holbrooki	Yes
Silurus glanis	Yes
Reptiles	
Chrysemys picta	No
Trachemys scripta	Yes
Chelydra serpentina	No
Macroclemys temminckii	No
Amphibians	
Rana catesbeiana [Lithobates catesbeianus]	No
Birds	
Oxyura jamaicensis	Yes
Mammals	
Myocastor covnus	No
Ondatra zihethicus	No
Castor fiber	No
Castor canadensis	No
Mustela vison	Yes
Procyon lotor	No

prevent new introductions or further spread in the territory of the EU, even if they are already present in some territories. According to this legislation it is also necessary to promote early detection and rapid eradication of species where they are not yet present or not yet wide-spread. Of the fifteen freshwater species in the current EU list of invasive species of concern, seven occur in Portugal, although at different levels of invasion (Table 3). While one of these species is widespread through-out all the river basins (*P. clarkii*) making control actions unfeasible, other species are still in initial stages of invasion, with only a few sightings every year (e.g. *Threskiornis aethiopicus* and *Oxyura jamaicensis*) or are still restricted to one or a few river basins (e.g. *E. sinensis* and *P. leniusculus*). Therefore, it may be viable to prevent or at least contain their spread. An intermediate status is attributable to *T. scripta* and to *Alopochen aegyptiacus*, both with a wider distribution and with reported reproduction in the wild.

Within the same EU list, three species are still absent in Portugal but may be introduced in a very near future, requiring urgent preventive actions (rank order 4 and 3, Table 3). These three species include: *Procambarus fallax*, a parthenogenetic crayfish currently sold in Portuguese pet shops (Anastácio pers. obs.); *Lithobates catesbeianus*, an amphibian which had a production farm near the Portuguese border (no longer in operation) and *P. parva*, a fish currently occurring in <100 km away from the Portuguese border in the Guadiana basin (Morcillo et al., 2017).

There are significant differences between the European and the Portuguese lists of invasive species. In what concerns freshwater fauna, seven of the 15 species in the EU list are not mentioned in the Portuguese legislation and at least four of these species have high rank orders in terms of likelihood of introduction or spread. Therefore, we strongly recommend a correction of the Portuguese list with the inclusion of *A. aegyptiacus, T. aethiopicus, P. fallax* and *P. parva*.

9. Conclusions

In this review we compiled for the first time the list of non-native faunal species in Portuguese freshwaters, briefly discussing the history of introductions since 1800 and identifying the most probable vectors of introduction and their regions of origin. We also reviewed the existing knowledge about ecological and economic impacts, invasion risk and potential distribution of invaders, under present and future climatic conditions. Furthermore, we identified non-native species for which strong management measures are recommended, while also highlighting gaps in the national legislation and in the information regarding some non-native faunal groups. For example, records of nonnative freshwater insects are almost absent from the literature, except in what concerns mosquito vectors of human diseases. It is also clear that there are numerous studies about ecological impacts but few concerning social or economic impacts of invaders.

Based on the available published data and ignoring the lists available in the legislation, we identified several invasive species which are likely to become established in a near future and are also likely to cause significant ecological or economic problems. These species include D. polymorpha, P. canaliculata (or P. maculata), P. parva, M. anguillicaudatus and S. erythrophthalmus. However, it is legally mandatory to act upon several freshwater species in the EU list of invasive species and some of these are not vet included in the national legislation. Our study highlighted that ten species in the EU list are of high national concern, namely P. clarkii, T. scripta, E. sinensis, P. leniusculus, A. aegyptiacus, O. jamaicensis, T. aethiopicus, P. fallax, L. catesbeianus and *P. parva*. This is because of being already present in Portugal or due to a high likelihood of introduction in a very near future. For these species it is essential to legislate, to plan, to implement or to continue with management actions devoted to prevention, early detection and control and containment. In the future, public awareness actions and education regarding species introductions is needed as well as more political compromise to tackle with this problem.

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Table 3

Ranking the likelihood of introduction or spread to the Portuguese mainland territory of freshwater species listed in the updated EU list of invasive species. The rank number 5 corresponds to a species already detected in Portugal. Rank 1 is the lowest likelihood value.

Species	Group	In Portugal			In Spain?	Kms to border	Rank
		Legislation?	In wild?	Captive populations?			
Procambarus clarkii	Crustaceans	yes	Yes	Yes	Yes	0	5
Trachemys scripta	Reptiles	Yes	Yes	Yes	Yes	0	5
Eriocheir sinensis	Crustaceans	Yes	Yes	No	Yes	0	5
Pacifastacus leniusculus	Crustaceans	Yes	Yes	No	Yes	0	5
Alopochen aegyptiacus	Birds	No	Yes	No	Yes	0	5
Oxyura jamaicensis	Birds	Yes	Yes	No	Yes	0	5
Threskiornis aethiopicus	Birds	No	Yes	No	Yes	0	5
Procambarus fallax	Crustaceans	No	No	Yes	No	500 + km	4
Lithobates catesbeianus	Amphibians	Yes	No	No	Yes	0–100 km	3
Pseudorasbora parva	Fish	No	No	No	Yes	0–100 km	3
Orconectes limosus	Crustaceans	No	No	No	Yes	100– 500 km	2
Myocastor coypus	Mammals	Yes	No	No	Yes	100– 500 km	2
Ondatra zibethicus	Mammals	Yes	No	No	Yes	100– 500 km	2
Orconectes virilis	Crustaceans	No	No	No	No	500 + km	1
Perccottus glenii	Fish	No	No	No	No	500 + km	1

Grey colored cells indicate the most serious type of situation when only two options are availabe (yes or no). In the rank collumn, the red is the most serious situation and light yelow is the situation of least concern.

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