Human Dispersal of Freshwater Invasive Fauna

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”Cause it’s a bittersweet symphony, this life (…) you’re a slave to the money then you die.”

“ There ain't no space and time to keep our love alive. We have existence and it's all we share”

“Oh, you're too afraid to touch. Too afraid you'll like it too much.”; “ ’Cause life's an ocean too much commotion, too much emotion. Dragging me down.”; “Sinking faster than a boat without a hull”

“Imagine the future, woke up with a scream. I was buying some feelings from a vending machine”; “She calls me, calls me the sun the sea” “One way to go (…) I don’t care what I find” “But how many corners do I have to turn?(...)Happiness coming and going (…) Well, I’m a lucky man” “a man called Sun I think my journey's just begun. (…)
Endless life….”

Ita sabes uma coisa…?

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Abstract

The main goal of this thesis was to improve the knowledge on the mechanisms involved on Human dispersal of freshwater invasive fauna, contributing for the management of these problematic species. Several vectors were investigated, both accidental and intentional, from a freshwater invaders list that included some of the worse species. It was found that the red swamp crayfish and the signal crayfish presented desiccation survival capacities compatible with long-distance human-mediated dispersal. Off-road vehicles constituted a viable accidental vector for invasive macroinvertebrates, like the red swamp crayfish and the bladder snail. Live bait capture using dip nets and crayfish trapping constitute viable vectors for invasive freshwater macrofauna dispersal. The former is more related with accidental transport of small invasive organisms, and the latter is mostly related with intentional transport of invasive fish species. The importance of the angling web forums as a useful tool to help detection of non-native fish species was demonstrated with the first record of European Perch, a non-native fish in continental Portugal. Freshwater anglers from Portugal and Spain presented preference for invasive fish species, similar mobility, low incidence of live bait use and similar perception of biological freshwater invasions processes and impacts. Differences among countries were found for angler's activity patterns throughout the year and motivations for introductions. Zebra mussel larvae desiccation survival is compatible with long-distance overland dispersal. Its transport by natural vectors, like ducks, or human vectors like fishing tackle, such as waders and keep nets is viable. Yet, when comparing both types of vectors, fishing tackle presented a higher propensity to spread zebra mussel larvae than ducks.

Dispersão de fauna invasiva dulçaquícola pelo Homem

Resumo

O principal objetivo desta tese foi melhorar o conhecimento dos mecanismos envolvidos na dispersão de fauna dulçaquícola invasiva pelo homem, contribuindo assim para a gestão destas espécies problemáticas. Investigaram-se vários vetores, quer acidentais quer intencionais de uma lista de invasores dulçaquícolas que incluem algumas das piores espécies. Verificou-se que o lagostim vermelho e o lagostim sinal possuem uma capacidade de sobrevivência à dessecação compatível com a sua dispersão a longa distância pelo Homem. Os veículos todo-o-terreno constituem um vetor viável para macroinvertebrados invasivos como o lagostim vermelho e o caracol aquático. A captura de isco vivo com recurso a camaroeiro e o uso de armadilhas para a captura de lagostim constituem vetores viáveis de dispersão para a macrofauna dulçaquícola, sendo que o primeiro está relacionado com o transporte acidental de pequenos organismos invasores e o segundo com transporte intencional de peixes invasores. Através do primeiro registo em Portugal Continental de Perca-europeia, uma espécie não-nativa, demostrou-se a importância de fóruns on-line de pesca desportiva como uma ferramenta útil para a deteção de peixes não-nativos. Os pescadores dulçaquícolas de Portugal e Espanha apresentam preferência por espécies de peixes invasores, similar mobilidade, baixa incidência no uso de isco vivo e similar perceção dos processos e impactos das invasões biológicas dulçaquícolas. Detetaram-se diferenças entre países nos padrões de atividade dos pescadores durante o ano e na motivação para as introduções. A sobrevivência à dessecação de larvas de mexilhão-zebra é compatível com o seu transporte a longas distâncias fora de água, sendo viável o seu transporte quer por vetores naturais, como patos, quer humanos como equipamento de pesca, como botas altas e redes de retenção. No entanto, quando se comparam ambos os tipos de vetores, o equipamento de pesca apresenta maior propensão que os patos para dispersar larvas de mexilhão-zebra.

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Chapter 1 - General introduction

1.1 Dispersal process and Man

Dispersal is the movement of organisms or propagules between spatially discrete localities or populations (Bilton et al., 2001), being the main process influencing the dynamics and evolution of populations and species (Mayr, 1963). Dispersal defines the spatial limits for colonization of new sites (Cáceres and Soluk, 2002) and influences the probability of extinction (Vos et al., 2001). Aquatic species can disperse naturally between water bodies, crossing inhospitable terrestrial landscape in two ways: by active dispersal, i.e. self-generated movements of individual organisms, both flying (e.g. insects, (Freeman, 1945)) or walking (e.g. crayfish (Cruz and Rebelo, 2007; Ramalho and Anastácio, 2015)), or through passive dispersal using an external agent. The external agents can be animal vectors, like birds (Anastácio et al., 2014; Banha and Anastácio, 2012; Rachalewski et al., 2013), mammals (Vanschoenwinkel et al., 2009; Waterkeyn et al., 2010a), or physical agents such as the wind (Brendonck and Riddoch, 1999) or water flow (Griffith et al., 1998). Nevertheless, introduction into a new aquatic habitat, across the geographic barriers that limit the native range, is normally done through human activities or human-mediated dispersal (Cohen and Carlton, 1998; Ricciardi and MacIsaac, 2000). Compared to natural range expansion, human-mediated dispersal occurs at a massively higher rate and with different geographic patterns; being a much more diverse and dynamic process (Lockwood et al., 2007). This process can be classified as intentional, when introductions are directly linked with human well-being, like food, game, environmental enhancement, bio-control, conservation and science; or accidental, as a secondary result of the intentional transfer of products, goods, services or other non-native species, when invaders are transported as hitchhikers or stowaways (EEA, 2012; Lockwood et al., 2007).

1.2 Non-native species and invasive species

By definition, a species that is introduced outside its native distribution range by human intervention, either directly or indirectly, is considered a non-native species (EEA, 2012; Lockwood et al., 2007). However, a continuous debate has been carried out by invasion ecologists, due to an ambiguous use of terminology; several terms like alien, invasive, exotic, weed, and others are used to define the concept of "non-native species"; but with an associated negative lexical context (Colautti and MacIsaac, 2004). Ironically, the concept of native species itself was re-analyzed, since humans have been transporting species for tens of millennia across the Late Quaternary. A new lexicon to explore the space between "native" and "non-native" has been developed, since some old introduced species have, nowadays, conservationist importance, being well implanted in their ecosystems, presenting a recognized ecological, social-cultural and iconic value. The importance of defining the term "native species" is of more than semantic interest. It is a vital part of modern ecology and conservation to make informed decisions on management, with eradication and restoration plans depending on it (Crees and Turvey, 2015). Like the two terms analyzed before, the term "invasive non-native species", was also subjected to lexical and definition debate. For Colautti and MacIsaac (2004), it reflects the biological invasion process. In this context, a non-native species becomes an invasive species after passing several "steps" or "filters" such as propagule pressure; physicochemical requirements of the potential invader and community interactions. As a result, if a non-native species passes these multiple biotic and abiotic filters, it may become widespread and dominant. Consequently, some species have the capacity to promote impacts perceptible by Man. This is the basis for the simplest definition of an "invasive non-native species" - a non-native species that has demonstrable ecological or economic impact (Lockwood et al., 2007).

1.3 Invasive species impacts

A global biological homogenization is under way because of the continuous spread of non-native invasive species (McKinney and Lockwood, 2001) as a result of human population geography at a broad spatial scale (Olden and Rooney, 2006). Numerous studies in several taxonomic groups and areas of the world showed the same picture - the number of non-native species established has been increasing, and accelerating in the last decades (Cohen and Carlton, 1998; Frank and McCoy, 1993; Pyšek et al., 2003) due to an increasing global trade (Levine and D'Antonio, 2003). The growing human population, associated with urbanization of landscape are the drivers of environmental degradation and introduction of non-native species is considered a major threat to native species persistence (Vitousek et al., 1997; Wilcove et al., 1998).

Freshwater ecosystems are limited in area, less than 1% of earth surface, but are one of the most important biodiversity reservoirs and are, due to its richness, space limitation and isolation, the most affected by biological invasions (Costanza et al., 2006; Dudgeon, 2014; EEA, 2012). In fact, in terms of economic valuation of biomes, freshwater ecosystems are 34 times more valuable than terrestrial systems per unit area (Costanza et al., 2006). These ecosystems are also subjected to anthropogenic disturbance due to the growing human demand for resources (Vörösmarty et al., 2010) which may favor the invasion process (Davis et al., 2000). In these ecosystems competition, predation and transmission of diseases between invasive species and native species are frequent and can pose a major threat to the latter (EEA, 2012; Lodge and Shrader-Frechette, 2003; Sala et al., 2000). Consequently, a general concern about invasions is spread between scientists and general public. However, the assumed importance of the invaders in causing widespread extinctions has been challenged, by some authors (Gurevitch and Padilla, 2004) but confirmed by others using the same data (Clavero et al., 2005). In spite of this discussion, the colossal economic losses associated with invasive species are very clear. In each year in Australia, Brazil, India, South Africa, the United Kingdom and the United States these have been calculated in approximately 300 billion USD (Pimentel et al., 2001; Pimentel et al., 2005). For Europe, the annual economic costs of this problem are estimated to be at least 12 billion EUR (Kettunen et al., 2009). The economic impact is not only restricted to losses, but also investment in control and prevention. As an example in the United states, the US Fish and Wildlife Service spends 825 000 USD per year (Leung et al., 2002).

So far, only a small proportion of non-native species invasions gave rise to hyper-successful nuisance species (Williamson and Fitter, 1996). Some of these can in fact have a few benefic or positive impacts, e.g. as food source for native species (Carlsson et al., 2009) or even Human benefits, supplying food or other services (Byers, 2002; Pimentel et al., 2002). Actually, the same species may have significant effects in some areas and negligible ones in others (Byers, 2002). Moreover, the perception of impact is subjective, since it suffers scientific and societal influences. The perception itself will also depend on a variety of ecological factors that determine the level of impact. Therefore, it is not obvious to determine when a non-native species has no impact (Lockwood et al., 2007). Summing up, according to the biological level of organization, the ecological impacts of invasive species can be classified into genetic, individual-level, population, community, ecosystem and landscape, regional and global impacts. Alternatively, a more general classification grouped invasive species impacts into 4 classes - biodiversity, ecosystem services, human health and economic activities (EEA, 2012). Regardless of the adopted classification it is obvious that what affects one type or level of organization will often affect others. Studying or quantifying how a given impact at a certain level translates its impact through the other levels is not an easy task (Lockwood et al., 2007).

1.4 Invasive species control and dispersal

Severe impacts require prompt management decisions and this often leads to removal with the purpose of eradication. Nevertheless, this is very expensive and, even more important, it has a very low success rate (Moyle and Light, 1996; Simberloff, 2001, 2003). Consequently, the study of dispersal mechanisms has been gaining importance, since it allows acting preventively on vectors and pathways to inhibit the undergoing invasion process (Bohonak and Jenkins, 2003; Colautti et al., 2005; Crooks and Soulé, 1999). From an economical perspective, post-invasion actions are less advantageous than prevention. There are higher costs in control and eradication of invasive species, but also in native species conservation (e.g. re-introduction actions) (Allendorf and Lundquist, 2003; Brooks et al., 2006; Leung et al., 2002). However, the amount of money allocated for prevention has been insufficient (Leung et al., 2002). Recently this perspective has changed with an increase in the attention given to preventive measures. In this context the European Union has defined a "EU Biodiversity Strategy to 2020", in which "Invasive non-native species and their pathways are identified and prioritized, priority species are controlled or eradicated, and pathways are managed to prevent the introduction and establishment of new invaders" (EEA, 2012).

1.5 Human mediated dispersal mechanisms of freshwater fauna

According to Mack (2004), a dispersal vector is the mechanism transporting species along a pathway, and a pathway is the route between the source region of a non-native species and its release location. The importance of a vector for a species will depend on the stage of the life cycle being transported, the number of surviving individuals transported per dispersal event, the frequency of such events, and the spatial patterns of vector movement (Johnson and Padilla, 1996). Preventing invasion at all sites is not feasible, but not all sites are vulnerable to invasion and this is essential to determine priority regions. Additionally, the diversity of vectors and pathways is immense and only a few have been extensively studied. However, for the extensively studied vectors, the species transported, as well as their origin or destination and their temporal variations are difficult to predict (Lockwood et al., 2007).

As previously exposed (section 1.1), human dispersal mechanisms can be divided into intentional or accidental. In freshwaters, intentional introduction is the most important dispersal process for vertebrates, especially for fishes, while for invertebrates the majority of the introductions are accidental (García-Berthou et al., 2007). The intentional transport of freshwater fauna, considering the FAO database - DIAS, plays the main role (over 70%) in species establishment over their native range (Welcomme, 1991). According to the same database, in fact the 10 most frequently introduced aquatic species in the world, are all freshwater fishes that have been introduced over 50 countries (García-Berthou et al., 2005). Many of these fish species were introduced worldwide as important aquaculture food items, with subsequent escape or direct release into the wild, as in the case of the channel catfish (*Ictalurus punctatus*) (Elvira and Almodóvar, 2001; FAO, 2015). Some invertebrates like crayfishes, were also introduced due to its importance as food, for example the Red swamp crayfish *Procambarus clarkii* (Hobbs et al., 1989).

Another important cause of intentional introduction of freshwater species has been sports fishing or angling importance, as is the case of the rainbow trout (*Oncorhynchus mykiss*), introduced in more than 100 countries (Lever, 1996). Many of these introductions were governmental, but in the last decades illegal introductions gained importance (Elvira and Almodóvar, 2001). The introduction of fish game species has been associated with the intentional introduction of other species, mainly other fishes, as forage species (Bote et al., 2004; Elvira and Almodóvar, 2001), or live bait (Ludwig Jr and Leitch, 1996). An alternative source of non-native freshwater species introductions is aquarium trade. Many species of fish (Duggan et al., 2006), aquatic turtles (Salzberg, 1995), but also invertebrates, like crayfish (Chucholl,

2013) and others have been introduced by pet owners into the wild. A less common reason for intentional introductions is bio-control. In freshwater ecosystems one the most iconic invasive species is the Mosquitofish, *Gambusia spp.,* introduced to control mosquitoes, which are vectors of malaria (Pyke, 2005). The main drivers of intentional introductions are historical and sociological factors. Most intentional introductions have written records of when and where they occurred (Lockwood et al., 2007). Therefore most vectors and pathways are well identified (García-Berthou et al., 2005; McDowall, 2004) and governments have imposed legislation to prevent those introductions.

Contrary to intentional introductions, in accidental introductions some aspects or characteristics of the potential invader are crucial. Firstly, its size is determinant since only "anything small enough to escape the notice" could be transported unintentionally (Lockwood et al., 2007). Indeed, the small size may also favor the transport process itself. Some transport by human vectors involves adhesion to objects, being ruled by the same physical principles as for passive dispersal by animals (Banha and Anastácio, 2012; Bie et al., 2012). Additionally, the organism's tolerance to different stressors (temperature, oxygen concentration, salinity and pollution) during the transport is also decisive. For example, when the transport occurs out of water, the desiccation survival capacity is decisive (Anastácio et al., 2014; Banha and Anastácio, 2012; Figuerola and Green, 2002). An organism with higher resistance to desiccation during transport, having a higher number of individuals transported alive, i.e., higher propagule pressure, results in a higher probability for successful introduction in new territories (Colautti et al., 2006).

Possibly, the most important difference between accidental and intentional transport is the fact that accidental processes involve much more diverse vectors, with almost unlimited mechanisms (Carlton, 1993). Therefore, it is much more difficult to evaluate accidental transport in terms of its overall importance, but evidence suggests that it is quite prevalent (Carlton, 2000). One of the most recognized accidental vectors is ship ballast water (Mack, 2004; Panov et al., 2009). In this type of vector, freshwater species may be “captured” in one continent, when ballast tanks are filled with water; cross an entire ocean and be released in a different continent. This process occurred for many European species of amphipods, zooplankton, mussels and fishes that invaded the North American Great Lakes (MacIsaac et al., 2002).

Commercial shipping and recreational boating constitute important vectors but with a more restricted area of influence in terms of transport distance, acting at a continental scale (Johnson et al., 2001). Species can be transported by these vectors in many different ways. Some dispersal mechanisms involve transport out of water with the organisms attached to boat surfaces, motors, anchors or material snagged by the anchor or aquatic macrophytes entangled on the boat trailer during retrieval of the boat. Other processes involve transport in water such as bilge water, engine cooling water or boat live wells (Johnson and Carlton, 1996).

Not only ships or boats move between waterbodies or watersheds. In theory any material that contacts with water and transits between different places or any mechanism that transports water between places can disperse small invasive species. Terrestrial vehicles, namely off-road vehicles or agriculture machines, sometimes cross freshwater ecosystems. The use of off-road vehicles is associated to many human activities such as irrigated agriculture, cattle farming, hunting, and many others. Waterkeyn et al. (2010b) demonstrated off-road vehicle dispersal of resistant freshwater invertebrate propagules of *Artemia* spp., large freshwater branchiopods, Cladocera, Ostracoda, Rotifera, Turbellaria, and Nematoda. Consequently, it is imperative to thoroughly study these possible vectors for invasive fauna.

Many recreational activities are directly linked to freshwater ecosystems such as kayaking, canoeing, shelling, and many others, but few of these have been studied as vectors for invasive fauna. For example, the role of scuba-diving wetsuits in spreading invasive aquatic species has already been proposed (Holeck et al., 2004; MacIsaac et al., 2004) and constitutes a viable vector for invasive amphipods (Bacela-Spychalska et al., 2013). Angling or sport fishing are not only responsible for intentional introductions. Anglers may accidentally disperse small invasive species in bait bucket water (Binimelis et al., 2007; Goodchild, 2000). However, any angling gear - like boots or waders - may transport invasive organisms (Gates et al., 2008). To assess the role of the anglers on both intentional and accidental introductions, it is decisive to study their habits and their perceptions regarding biological invasions. The obtained information will allow implementing correct restrictive legislation and correct awareness programs. In spite of this, until now not many studies on this subject have been implemented (Gozlan et al., 2013; Keller et al., 2007; Kilian et al., 2012; Lindgren, 2006). Most of these works studied only a particular aspect (e.g. livebait use), at a regional scale, mainly on the USA.

Concluding, "Human dispersal of Freshwater invasive fauna" is a huge field of research, especially unintentional dispersal mechanisms, since many mechanisms are still poorly studied. Additionally, it is important to study the motivations of intentional introductions and the perceptions of impacts, in order to implement correct awareness programs to the group of people involved in these processes (e.g. anglers).

1.6 Aims and structure of the thesis

Taking into account the importance of improving the knowledge in the area of Human dispersal of freshwater invasive fauna, as previous exposed, the main goal of this thesis is to study dispersal mechanisms and decisive factors for this process. It is also an objective of this thesis to obtain relevant information for vector management and invasions prevention by government and management agencies connected with water uses.

In the six following chapters (chapter 2 to 7) the research conducted is described in detail. Each chapter corresponds to a scientific research paper that was published in an international peer-reviewed scientific journal, except chapters 6 and 7 that are still under revision.

Chapter 2 investigates the desiccation survival capacities of two highly invasive crayfish species. The desiccation survival is decisive both for accidental and intentional Human assisted dispersal. Laboratorial experiments were conducted in order to evaluate this capacity during summer conditions.

In Chapter 3 the viability of off-road vehicles for accidental transport of invasive species was assessed. This was studied for an aquatic snail and one crayfish species. In fact, the desiccation survival capacity of crayfish, studied in the previous chapter, constitutes a decisive factor for this type of vector, because the transport occurs out of water. This work consisted in a sequence of experiments analyzing successive steps of the transport process, namely the adhesion and permanence on the vector, the transport survival and finally the release into the receiving environment.

In Chapter 4, the live bait capture and crayfish trapping potential as vectors for freshwater invasive fauna was studied. In the Tagus river basin (Portugal), where both activities are common, we evaluated the probability of capture and the electivity of the local aquatic macrofauna according to the method used. During the compulsory removal of the invasive species captured we also quantified fish desiccation survival.

Chapter 5, reports for the first time the presence of a non-native fish, the European Perch (*Perca fluviatilis*), in continental Portugal. The presence of this species was first reported on an angling web forum and its occurrence was scientifically confirmed later in a small reservoir of the Tagus river basin, located in the central region of Portugal. The importance of the angling web forums as a useful tool to help detection of non-native fish species introductions, and its potential for education on biological invasions, is discussed.

In chapter 6, the Iberian freshwater angler's habits and perceptions related to biological invasions were studied, using an international and bilingual survey in Spain and Portugal. Important aspects, such as angler's activity and mobility, were assessed, as well as the frequency of risk behaviors such as live bait use. The perception of introductions' impacts, motivation for introduced species in aquatic ecosystems and fish species preference was analyzed since it is decisive for awareness campaigns.

Finally in chapter 7, zebra mussel larvae dispersal by fishing gear (waders and keep net) versus mallard ducks was evaluated and compared using an experimental approach under field conditions. For this purpose, the zebra mussel larvae adherence and survival rate on each vector was studied. In addition, zebra mussel larvae desiccation survival under a set of controlled temperature and humidity conditions was evaluated.

The general conclusions are presented in Chapter 8, which includes a discussion of the main results and implications of this thesis and outlines future perspectives. Advices or suggested measures for invasive species management are also presented under this chapter.

Chapter 2 - Desiccation survival capacities of two invasive crayfish species.

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Desiccation survival capacities of two invasive crayfish species

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ABSTRACT

The signal crayfish, *P. leniusculus* and the red swamp crayfish, *P. clarkii* are two invasive crayfish species with widely world distribution, being both present at Iberian Peninsula. In this work we study the desiccation survival capacities of both species at 24 ◦C. Our results showed that both species are capable of surviving exposure to air for long periods of time, with an LT_{90} of 17.6 and 21.5 h, respectively, for red swamp crayfish and signal crayfish. Our findings are in accordance with the great overland dispersal capacities attributed to these crayfish species.

Key-words: *Pacifastacus leniusculus*, *Procambarus clarkii*, desiccation survival, invasive species, dispersal

Crayfish are one of the most commonly introduced freshwater organisms and are responsible for declines and extinctions of native species throughout the world (Guan and Wiles, 1997; Lodge et al., 1998, 2000; Nyström et al., 2001; Westman et al., 2002). The signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and the red swamp crayfish, *Procambarus clarkii* (Girard, 1852) are two North America native crayfish species with worldwide distribution due to their economic importance, but also due to their high dispersal abilities, fast growing populations and wide niches (Hobbs et al., 1989). Severe negative impacts on invaded areas are attributed to these two crayfish species, such as competition with native species (Nyström, 1999), dissemination of the crayfish plague (Diéguez-Uribeondo et al., 1997), habitat and ecosystem changes (Geiger et al., 2005), negative effects on amphibian populations (Nyström, 1999; Cruz et al., 2008) and losses on economic activities such as rice production (Anastácio et al., 2005). In the Iberian Peninsula these two crayfish were first introduced into Spain nearly at the same time, *P. clarkii* in 1973 and *P. leniusculus* in 1974 respectively, for aquaculture purposes (Diéguez-Uribeondo et al., 1997; Alonso et al., 2000). In Portugal, the fist records of these species were in 1979 for *P. clarkii* in the Caia river (Ramos and Pereira, 1981) and in 1997 for *P. leniusculus* at the river Maçãs (Bernardo et al., 2001). Currently these species have a wider distribution, with *P. clarkii* being present across all the Iberian Peninsula, and *P. leniusculus* having a more restricted distribution. The present distribution of these species reflects their high dispersal capabilities, high population growth rates and wide niches, which makes them very successful invaders (Hobbs et al., 1989) but also years of successive illegal translocations (Alonso et al., 2000; Diéguez-Uribeondo, 2006).

The desiccation survival capacities of aquatic species are intrinsically related with their passive dispersal capacities (Figuerola and Green, 2002), but also with their active dispersal capacities on dry land (Correia and Ferreira, 1995; Cruz and Rebelo, 2007). In this work, we assess the desiccation survival capacities of *P. clarkii* and *P. leniusculus*.

Red swamp and Signal crayfish were collected using baited traps, respectively in the Divor (38◦ 52’56.58’’N; 8◦10’.21.84’’W) and the Maçãs rivers (41◦40’36.00’’N; 6◦ 38.06’.00’’W). Specimens of both species were separately acclimated during 5 days in aerated plastic containers (60 × 40 × 40 cm) with dechlorinated tap water at an 12:12 h light:dark cycle and carrots were fed to both species. We performed two separate laboratory experiments to check how long each of these two invasive crayfish could survive out of water, under controlled conditions at 24 ◦C. This temperature was chosen because it falls on the top of the range for the annual average maximum temperatures in the Iberian Peninsula (Ninyerola et al., 2005). Eighty-one red swamp crayfish (cephalotorax length = 44.36 mm ± 4.53 SD; weight = 19.38 g ± 6.63 SD; Fulton index = 0.23 ± 0.03 SD) and Signal crayfish (cephalotorax length = 37.88 mm ± SD; weight = 16.64 g ± 7.90 SD; Fulton index = 0.29 ± 0.08 SD) were distributed into 9 groups of 9 individuals for each species. Each crayfish was individually placed into a plastic box (20 × 20 cm) and the boxes were kept at 24 ◦C with a relative humidity (RH) of 30% and 50% for the experiments with the red swamp crayfish and the signal crayfish respectively. The experiments lasted 27 h and every 3 h, the number of crayfish alive in one randomly selected group was checked. Crayfish were considered alive if we could detect movement of pereiopods, antenulles or maxillipeds. This procedure started by visually searching, during 30 s, for the presence of movement. If no movement was detected the individual was removed manually from the box, holding it by the pleon on an “upside down” position. Under these circumstances, many of the alive crayfish presented a reflex movement of pleon, if they did not we would then watch more closely for vestigial movement of pereiopods, antenulles or maxillipeds. Also, alive individuals in this position presented the pereiopods flexed and dead ones presented all pereiopods downturned by gravity. Statistical analyses were performed using IBM SPSS Statistics 20.

Our results show that both species presented no mortality until a period of 3 h out of water. Red swamp crayfish started to die after 6 h of desiccation (Figure 1) but signal crayfish only started to die at 9 h (Figure 2). The number of crayfish alive was registered for a maximum of 18 and 21 h for red swamp and signal crayfish, respectively. Probit analysis indicated for red swamp crayfish an LT_{50} of 11.9 h (95% C.L. = 9.9−13.7) and an LT_{90} of 17.6 h (95% C.L. = 15.4−21.6) (Figure 1). The statistical model fitted adequately our observed data (Pearson goodness-of-fit test: X^2 = 1.289, d.f = 8, P = 0.996). For signal crayfish a probit analysis indicated an LT_{50} of 15.8 h (95% C.L. = 13.9−17.7) and an LT_{90} of 21.5 h (95% C.L. = 19.2−25.6) (Figure 2). The statistical model also fitted adequately our observed data (Pearson goodness-of-fit test: X^2 = 1.661, d.f. = 8, P = 0.990). Our study shows that both *P. clarkii* and *P. leniusculus* can survive out of water easily more than 10 h in severe conditions, which are typical of the Iberian summer. Although our findings show an apparent higher desiccation survival capacity of *P. leniusculus* face to the *P. clarkii*, the observed differences may simply reflect different conditions, namely the higher relative humidity (almost the double) during the *P. leniusculus* experiment. Under similar conditions, namely of temperature, other exotic freshwater crustaceans with a wide distribution across Europe, the Mediterranean river shrimp, *Athyaephyra desmarestii* (Banha and Anastácio, 2012) and the North American amphipod, *Crangonyx pseudogracilis* (Rachalewski, 2013) presented a survival capacity approximate 10 times lower than the presently studied species.

These findings have several implications for the dispersal of these crayfish species both by active and passive means, including Human mediated transport. For active dispersal, namely walking over dry land, a great capacity to survive out of water means a great capacity to cover large distances out of their natural environment. According to Pond (1975) the white-clawed crayfish (*Austropotamobius pallipes*), a native European crayfish species, presented a mean walking speed on dry land of 54 m/h, so in 10 h a crayfish would walk 540 m.

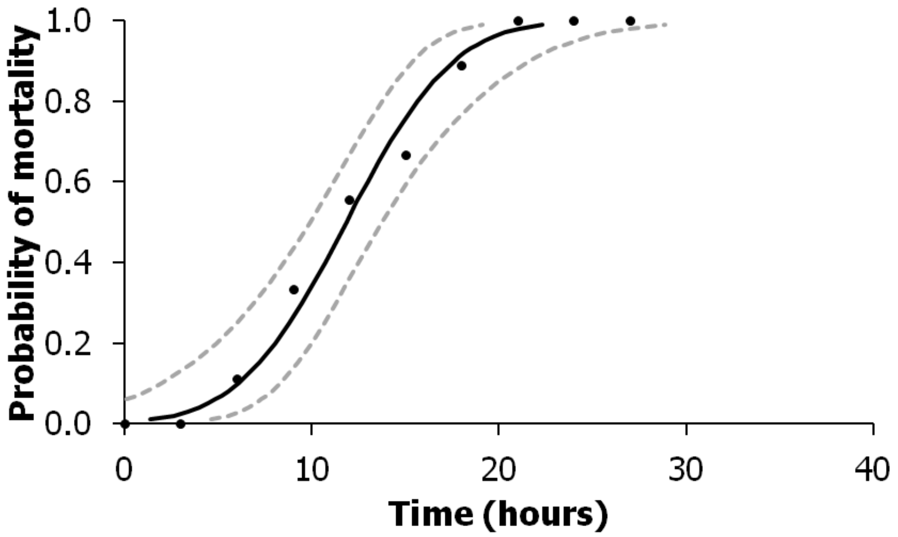

Figure 1

Probability of *P. clarkii* mortality as a function of the time spent out of water at 24 ◦C and 30% relative humidity. The black dots are the observed proportions of dead *P. clarkii*; the black line was obtained by Probit analysis, with the respective 95% confidence intervals (dotted line).

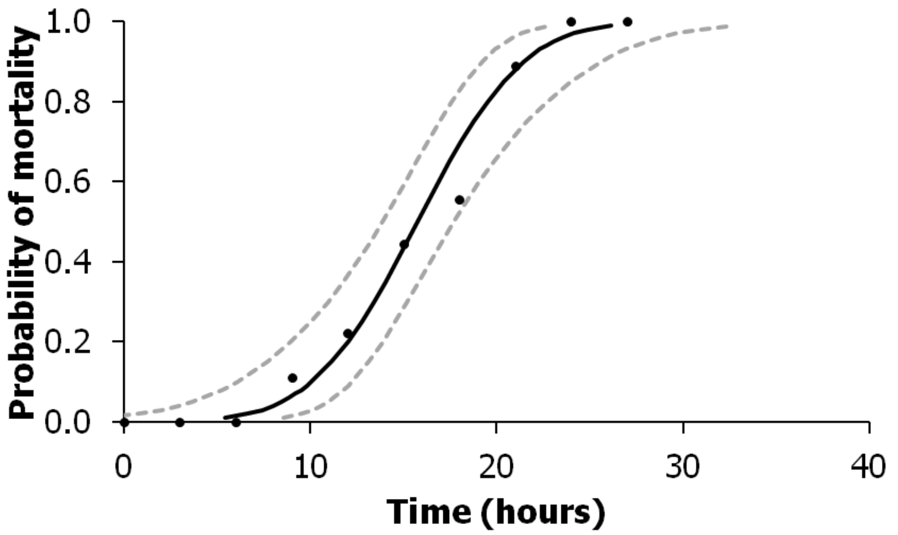

Figure 2

Probability of *P. leniusculus* mortality as a function of the time spent out of water at 24 ◦C and 50% relative humidity. The black dots are the observed proportions of dead *P. leniusculus*; the black line was obtained by Probit analysis, with the respective 95% confidence intervals dotted line).

For *P. clarkii* the maximum observed walking speed on dry land was of 90 m/h (Ramalho, 2012) although the mean values are lower. Taken our results for this species, namely the LT_{90} value, the maximum distance for active dispersal on dry land would be of approximately 1.6 km if walking continuously and always heading in one direction. This distance is enough to surmount natural or Human-made barriers on a stream, but also, under drought situations, it allows crayfish to escape from a drying pool and reach a more permanent water body. So, the desiccation survival capacities of *P. clarkii* and *P. leniusculus* are important features that can explain their great dispersal capacities. Our findings have also implications for passive dispersal, namely Human translocations; it is known that crayfish introductions and translocations that allowed many crayfish species to invade large geographic areas are due to illegal bucket transport by fishermen (Holdich et al., 2009; Lodge et al., 2000; Taylor 2000; Souty-Grosset et al., 2006). For a fisherman, the transport of crayfish specimens out of water is simpler and involves less equipment, but it also allows the transport of more individuals from one point to another. Therefore according to our results on the survival capacities of *P. clarkii* and *P. leniusculus*, both species can be easily transported for long distances in a small bucket or a cloth bag, undetected by the authorities. The distances involved in this process can easily overcome river basins, separated by dozens of kilometers if a car is involved. In conclusion, any management actions directed to these invasive species should take into account the great desiccation survival capacity of these invasive crayfish species.

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Chapter 3 - Dispersal of two freshwater invasive macroinvertebrates, *Procambarus clarkii* and *Physella acuta*, by off-road vehicles

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Dispersal of two freshwater invasive macroinvertebrates, *Procambarus clarkii* and *Physella acuta*, by off-road vehicles

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ABSTRACT

1. The red swamp crayfish (*Procambarus clarkii*) and the bladder snail (*Physella acuta*) are two invasive aquatic macroinvertebrate species with worldwide distribution that are plagues in rice fields.

2. The purpose of this study was to investigate the possibility of accidental human dispersal of these two species by off-road vehicles.

3. An experimental approach was used owing to the difficulty of obtaining field data on low-probability events such as passive dispersal. Experiments were performed testing the probability of attachment of both organisms to mudguards of off-road vehicles as well as successful transport and release into the receiver water body. Recently-hatched *P. clarkii* and all *P. acuta* sizes available in the field were used.

4. Results showed that *P. clarkii* and *P. acuta* can be transported in mud adhering to an off-road vehicle. No differences were found in the proportions of adhered organisms between species or for each species at the different densities used because of low overall retrieval. For *P. acuta*, the attachment probability varied between 0.5% both for 50 and 200 individuals m^{-2} and 1% for 100 ind. m^{-2}. For *P. clarkii*, no individuals adhered at 50 ind. m^{-2}, but the probabilities were 1% and 1.5% for 100 and 200 ind. m^{-2}, respectively.

5. A transport-survival experiment showed a mean survival distance of 83.2 km for *P. clarkii* and 92.2 km for *P. acuta* under dry conditions. Rain or wet conditions severely reduced successful transport.

6. The proportion of organisms falling into a receiving water body increased with the vehicle's speed while crossing the water.

7. Although the results demonstrated a low probability of occurrence of attachment, they also showed that, if attachment occurs, aquatic organisms have a high chance to be released successfully in a suitable environment after long-distance transport by vehicle.

KEYWORDS: Human-mediated dispersal; accidental introductions; biological invasions; vehicles; mud; freshwater invertebrates; *Procambarus clarkii*; *Physella acuta.*

INTRODUCTION

A global biological homogenization is under way because of the continuous spread of non-native invasive species (McKinney and Lockwood, 2001). This is one of the main global threats to biodiversity and a cause of animal extinction, also resulting in relevant economic and social damage (Moyle and Light, 1996; Pimentel et al., 2000; Clavero and García-Berthou, 2005). From a conservationist's point of view, this problem is especially important in freshwater environments since these habitats are one of the major biodiversity reservoirs (Costanza et al., 1997). It is known that, once an invasive species is established in a new habitat, eradication is difficult and rarely achieved, with highly expensive costs involved in the control actions (Moyle, 1999; Simberloff, 2001, 2003). Consequently, prevention is the main defence against biological invasions by acting on vector management. In this context, knowledge of the dispersal mechanisms is crucial (Crooks and Soulé, 1999; Bohonak and Jenkins, 2003).

Aquatic species can disperse naturally between water bodies by active dispersal, both flying (e.g. insects, Freeman, 1945) or walking (e.g. crayfish, Grey and Jackson, 2012) or even through passive dispersal using animal vectors such as birds (Banha and Anastácio, 2012), mammals (Vanschoenwinkel et al., 2008a; Waterkeyn et al., 2010a), or other agents such as wind (Brendonck and Riddoch, 1999) or water flow (Griffith et al., 1998). Nevertheless, introduction into a new aquatic habitat, across the geographic barriers that limit the native range or invaded range, is normally through human activities or human-mediated dispersal (Cohen and Carlton, 1998; Ricciardi and MacIsaac, 2000). Human-mediated dispersal of aquatic organisms can be intentional or accidental. For vertebrates, especially for fishes, intentional introduction is the most important dispersal process, while for invertebrates the majority of the introductions are accidental (García-Berthou et al., 2007). Several economic and recreational activities involving off-road motor vehicles, such as irrigated agriculture, cattle farming, hunting, and many others are closely associated with inland aquatic systems. These can act as possible vectors for invasive fauna, but have not been thoroughly studied. In spite of this, Waterkeyn et al. (2010b) showed evidence of off-road vehicle dispersal for resistant invertebrate propagules, including resting eggs such as Artemia spp., large freshwater branchiopods, Cladocera, Ostracoda, Rotifera, Turbellaria, and Nematoda. In motor-vehicle mediated dispersal, as in other types of passive dispersal of aquatic organisms involving adhesion (e.g. by animal vectors), the resistance to desiccation during transport (Figuerola and Green, 2002) and propagule size (De Bie et al., 2012) act as limiting factors. According to this idea, the aquatic organisms that are smaller, and resistant stages, such as resting eggs, which allow surviving extended periods of drought (Brendonck and De Meester, 2003; Vandekerkhove et al., 2013), are more apt to be successfully transported. However, a recent study by Banha and Anastácio (2012) showed that large macroinvertebrates, such as river shrimp (*Athyaephyra desmarestii*) without these adaptations can be transported successfully for long distances by waterbird ectozoochory. In addition, Bacela-Spychalska et al. (2013) found that human-mediated dispersal of killer shrimp (*Dikerogammarus villosus*) could also occur via scuba diving gear and boat ropes as vectors. Therefore, it is possible that this can occur with other macroinvertebrates and with other vectors such as off-road vehicles.

Rice fields are one of the most susceptible aquatic habitats to accidental invasion by aquatic invertebrates (García-Berthou et al., 2007). The reason for this is the intense circulation of vehicles, people, and materials within and across these areas. These artificial habitats occupy an area larger than any other crop in the world. In the Mediterranean region, rice fields have a special ecological importance because 80–90% of natural wetlands have disappeared and rice fields act as a substitute (Fasola and Ruiz, 1996). *Procambarus clarkii* (Girard, 1852) and *Physella* (=*Physa*) *acuta* (Draparnaud, 1805) are two invasive aquatic macroinvertebrate species with worldwide distribution and both often occur with high densities in these artificial wetland habitats (González-Solís and Ruiz, 1996; Suhling et al., 2000; Anastácio et al., 2005a, b). Several studies have been carried out in rice fields in the Iberian Peninsula on the ecology of both species, namely in the basins of the Sado (Cruz and Rebelo, 2007), Mondego (Anastácio and

Marques, 1995), Guadalquivir (Cano and Ocete, 1997) and Ebro (González-Solís and Ruiz, 1996; Oscoz et al., 2010). These studies demonstrated several adverse impacts on local biodiversity, especially for *P. clarkii*. In fact, crayfish invasion is responsible for the decline and extinction of native species throughout the world (Guan and Wiles, 1997; Lodge et al., 2000; Nyström et al., 2001; Westman et al., 2002; Almeida et al., 2013).

The arrival of *P. acuta* to Europe is attributed to an accidental introduction during the cotton trade between France and the United States (Anderson, 2003). The expansion of their invaded range is attributed to animal vectors, namely waterbirds and large mammals (Van Leeuwen et al., 2013). Unlike P*. acuta*, *P. clarkii* invasions are assumed to be the result of intentional introductions associated with aquaculture, legal or illegal stocking, live food trade, aquarium trade, and pond trade (Lodge et al., 2000).

For the management of invasive species, it is essential to know all processes implicated in their dispersal; therefore, dispersal by off-road vehicles needs to be investigated. Owing to the difficulties of obtaining field data on low-frequency events such as passive dispersal, an experimental approach was used to study the dispersal of *P. clarkii* and *P. acuta*. The aim was to evaluate the validity of off-road vehicle dispersal by quantifying: i – the probability of aquatic macroinvertebrate adhesion to the motor vehicle; ii – the probability of aquatic macroinvertebrate survival during transport; and iii – the probability of successful arrival at the receiving habitat.

METHODS

Experiments were performed to calculate the probability of the successful passive dispersal of invasive freshwater macroinvertebrate fauna by an off-road vehicle. The vehicle used was a Land Rover Defender 110 with RadialX® Atitude cross tubeless tyres. All the *P. acuta* and *P. clarkii* individuals used were caught in a rice-drainage channel near Salvaterra de Magos, River Tejo basin, Portugal (39°02′N, 8°44W) using a Wildco® triangular dipnet (1 mm mesh; 30 cm×30cm frame). These were kept for 2 days before the experiments in an aerated tank (57×43×39 cm) and fed with *Paspalum paspalodes* and *Cynodon dactylon* leaves collected in the same place.

Procambarus clarkii total length (TL) corresponds to body size and for *P. acuta* TL corresponds to total shell length. Both were measured with a digital caliper. Previous literature indicated a higher likelihood of transport for smaller organisms (Boag, 1986; Figuerola and Green, 2002; De Bie et al., 2012). Consequently, only recently-hatched *P. clarkii* were used. Since the experiments were not simultaneous, the availability of recently-hatched *P. clarkii* at the time of each experiment conditioned average size, but the smallest available cohort was always used. *Procambarus clarkii* size range was 6.12 to 12mm, corresponding to individuals less than 2weeks old. *Physella acuta* individuals used in this work comprised all cohorts available during late autumn (4.3 to 11.1mm).

Air temperature and relative humidity were measured during the experiments using a thermo-hygrometer, and the water temperature and dissolved oxygen were measured using a multiparameter probe (Multiline-WTW). Wind direction and velocity were taken from the nearest meteorological station (Geophysical Center, University of Évora). All experiments were performed in Évora during late autumn, in November and December.

Adherence to an off-road vehicle

An experiment was performed to check if *P. acuta* ($\overline{TL}$ = 7.57 mm ± 2.07 SD) and *P. clarkii* ($\overline{TL}$ = 8.43 mm ± 1.11 SD) can be transported in the mud attached to an off-road vehicle crossing a waterbody. A stream margin was simulated using an artificial pool (1 m wide; 0.5 m long; 0.2 m deep), with a plastic sheet (1.5 m × 1m) covering the bottom. The edges had a 45º inclination to minimize bumping of the vehicle and loss of attached sediment. The depression containing the pool was excavated with one shovel on a dirt road. The pool contained 0.004m^3

(0.05m thickness) of bottom substrate (clay loam soil, clay – 52.9%, silt – 32.5%, fine sand – 3.2%, coarse sand – 11.5%) taken from the site where the animals were collected, and water was added to 0.1m depth. This water depth was used because the snails (personal observation) and *P. clarkii* (Banha and Anastácio, 2011) can be found in very shallow waters and because the water depth in local rice fields, one of the most important habitats for these species, does not exceed 20 cm (Vergara, 1985; Anastácio et al., 1999). The dissolved oxygen content and water temperatures in the artificial pool (Table 1) were in accordance with the range of values found in the rice fields where the organisms were collected (Ramalho, 2012). Equal numbers of *P. acuta* and *P. clarkii* were placed into the pool. Three different densities that can be found in the field were tested: 50, 100, and 200 individuals m^{-2} for each of the species. *Procambarus clarkii* can reach 231 individuals m^{-2} in the rice field where they were collected (personal observation), but Harper et al. (2002) found crayfish densities greater than 500 individuals m^{-2}. *Physella acuta* can reach densities of more than 200 individuals m^{-2} in the same type of habitat (González-Solís and Ruiz, 1996; Suhling et al., 2000). The vehicle was driven at 15 km h^{-1}, which corresponds to the highest safe velocity to cross this pool. The vehicle was stopped 5m after crossing the depression with one wheel, and the substrate adhering to the mudguard was collected and sieved with an ASTME 11 sieve (1mm mesh). The number of animals transported was counted and their state (alive or dead) was registered. At the end of this process, the plastic sheet and its contents were removed. The whole process was repeated 200 times for each density. Fresh organisms were used in each replicate and a new pool was created. The organisms were held for a 5-min period of acclimatization in the pool before each replicate.

Transport survival experiment

An experiment was conducted to verify the capacity of the study species to stay on the substrate attached to a vehicle's mudguard and to survive the transport process. One hundred

P. acuta ($\overline{TL}$ = 7.98 mm ± 1.33 SD) and the same number of *P. clarkii* ($\overline{TL}$ = 8.42 mm ± 1.15 SD) were randomly distributed into 10 groups of 10 individuals. This number of individuals was much higher than the observed number of attached organisms per replicate in one of the previous experiments. However, it was assumed that, because of their small size, the organisms do not interfere with each other while attached and, therefore, a large enough sample would be obtained for each replicate. For each run of this experiment, 100 g of substrate was added to one mudguard. This value corresponds approximately to the average quantity of substrate adhered and transported in the previous experiment. One group of each species was randomly incorporated in the substrate. The vehicle was then driven for a predetermined distance at 90 km h^{-1} on a bumpy tar road with a QI (Quality index) of 2.37. The quality index was developed by Picado-Santos et al. (2006) and is based on the PSI (Present Serviceability Index) value (HRB, 1962) adopted by Sebaaly et al. (1996). The QI has a range from 0.0 to 5.0, with 0.0 being the worst and 5.0 the best pavement-conservation situation. The QI was obtained with Equation (1) and the following values: IRI (international roughness index) = 3500 mm km^{-1} (indirect estimation following Picado-Santos et al., 2006); the following values were measured in the field: Rt (mean rut depth in year t) = 16 mm; Ct (percentage alligator cracking area in year t) = 7 m^2 per 100 m^2; St (linear cracking area in year t) = 12 m^2 per 100 m^2; Pt (percentage patching in year t) = 53 m^2 per 100m^2.

$$IQ_t = 5 \times e^{-0.0002598 \times IRI_t / 2} - 0.002139 \times R_t^2 - 0.03 \times (C_t + S_t + P_t)^{0.5}$$

(1)

The road was dry, with no puddles. At the end of the trip, the mud that was still attached to the mudguard was gently removed and sieved through an ASTME 11 sieve (1mm mesh). The number of animals transported was counted and their state (alive or dead) was registered.

Individual *P. clarkii* and *P. acuta* were also considered 'dead' if they fell off the jeep. Ten different distances from 10 to 100 km, at 10 km intervals, were used randomly.

Experiment testing the success of the arrival process

An experiment was performed to evaluate the probability of the study species falling off the vector (substrate on the jeep's mudguard) into the receiving water body. Nine hundred *P. clarkii* ($\overline{TL}$ = 8.93 mm ± 1.00 SD) and the same number of *P. acuta* ($\overline{TL}$ = 8.12 mm ± 1.47 SD) were randomly distributed into 90 groups of 10 individuals of each species. As in the previous experiment, 100 g of substrate was added to the mudguard for each run of the experiment and one group of each species was randomly incorporated into the substrate. The vehicle was then driven across the test pool and was immobilized after 5 m. The test pool (3 m wide; 5 m long; 0.2 m deep) was created by modifying the shape, dimension and water depth of one natural stream. Once again, the mud that was still attached to the jeep's mudguard was removed and sieved and the number of animals transported was counted. In this experiment three different velocities, 10, 20 and 30 km h^{-1}, were tested. The third corresponds to the maximum velocity for safely crossing this stream. For each velocity the process was replicated 30 times.

Statistical analysis

The results for the adherence of organisms (*P. clarkii* and *P. acuta*) to the vehicle were analysed using a chi-square test for a contingency table. A Wilcoxon signed-rank test was used to check for differences between species. In order to assess the probability of trip survival of both species, a Kaplan–Meier analysis was used. A chi-square test for a contingency table was applied to the experiment to test the probability of falling into the receiving waterbody as a function of vehicle velocity and to check for differences between the number of animals that had fallen from the vehicle. All analyses were performed using IBM SPSS Statistics 20.

RESULTS

The environmental conditions during the experiments are shown in Table 1.

Table 1 Environmental conditions during the experiments.

Experiment	Air Temperature (ºC)	Water Temperature (ºC)	Relative Humidity (%)	Wind velocity (m s^{-1})	Dissolved oxygen (mg L^{-1})
Adherence	13.4 ±3.2 (9.8-20.7)	12.5 ±2.6 (7.5-15.3)	53.9 ±9.6 (40-70)	1.50 ±1.29 (0.2-4.2)	3.84 ± 0.44 (3.29-4.65)
Transport survival	11.9 ±1.0 (10-13.5)	-	78.6 ±8.9 (64-91)	1.77 ±0.41 (1.2-2.5)	-
Arrival	9.2 ±2.7 (6-13)	12.1 ±2.1 (9.2-14)	76.2 ±12.7 (56-91)	0.85 ±0.32 (0.5-1.4)	-

Experiment: Adherence — Adherence to an off-road vehicle; Transport survival — Transport-survival experiment; Arrival - Experiment testing the success of the arrival process. Mean, standard, and range of values are given.

Adherence to an off-road vehicle

The results show that the adherence of *P. clarkii* juveniles and individuals of *P. acuta* to motor vehicles crossing a water body is possible via the mud attached on the mudguards. A Chi-square test of independence on a contingency table showed that the three densities used had no effect on the proportion of individuals transported, either for *P. clarkii* (X^2 = 2.824; d.f. = 2; P = 0.244) or *P. acuta* (X^2 = 0.777; d.f. = 2; P =0.503). Since density had no significant effect on the proportions of individuals transported, a Wilcoxon signed-rank test was applied to detect differences between species, aggregating the data from all densities. This test showed no differences between *P. clarkii* and *P. acuta* (Z = –0.577; $P_{\text{2-tailed}}$ = 0.564; Monte Carlo $\text{Sig.}_{\text{2-tailed}}$ = 1.000 with 99% confidence interval = 0.996–1.000). For *P. acuta*, the results were an adherence probability of 0.5%, i.e. one individual in 200 trials for the densities of 50 and of 200 individuals m^{-2} and a probability of 1%, i.e. two individuals in 200 trials for 100 ind. m^{-2}. No *P. clarkii* were transported at 50 ind. m^{-2}, but the probability was 1%, i.e. two individuals in 200 trials and 1.5%, i.e. three individuals in 200 trials for 100 and 200 ind. m^{-2}, respectively (Figure 1). In this experiment, the mean weight of mud attached to the mudguard was 100.96 g ± 42.64 SD (15.37–

194.89 range). Considering all data from all densities, the probability of adherence for *P. clarkii* was 0.8% and 0.7% for *P. acuta*.

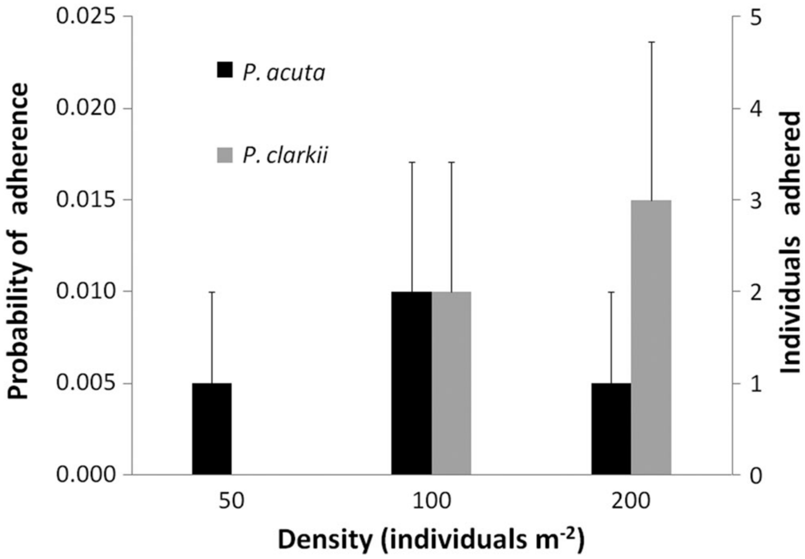

Figure 1. Probability of adherence and number of individuals (± SE) of *Physella acuta* and *Procambarus clarkii* adhered to one off-road vehicle's mudguard (number of transport events per vehicle crossing over the pool) at three different densities (n=200).

Transport survival experiment

The results show for both species that the probability of survival during transport is very high (close to 1) for distances reaching 30 km (Figure 2). The maximum distance tested was 100 km with results for *P. clarkii* and *P. acuta* showing a 30% and 90% rate of successful transport respectively (Figure 2). During all trials, including the maximum distance tested, the mud did not dry completely, always presenting a soft texture at the end of the trial. For the Kaplan–Meier analysis, data for the 40 km distance transport was excluded because rainfall (0.4mm) caused the loss of most of the mud. Kaplan–Meier analysis indicated a mean survival distance of 83.2

km (95% confidence interval: 78.1–88.3) for *P. clarkii* and 92.2 km (95% confidence interval: 88.3 – 96.1) for *P. acuta*.

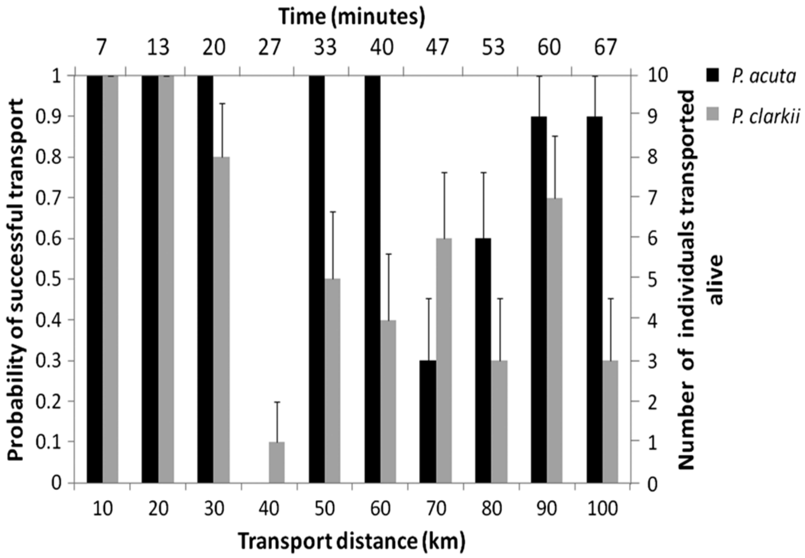

Figure 2. Probability and number of organisms (± SE) of *Physella acuta* and *Procambarus clarkii* transported on an off-road vehicle reaching the destination point and remaining alive, as a function of the distance travelled (n = 10).

Experiment testing the success of the arrival process

A Chi-square test of independence on a contingency table indicated that the proportion of organisms falling into the receiving water body was dependent on the vehicle's velocity, both for *P. clarkii* (X^2 = 76.567; d.f. = 20; P ≤ 0.001) and *P. acuta* (X^2= 76.810; d.f. = 20; P ≤ 0.001). The same test showed differences between species at a 10 km h^{-1} (X^2 = 22.289; d.f. = 9; P = 0.008) and 20 km h^{-1} speed (X^2 = 22.478; d.f. = 10; P = 0.013). However, for the highest speed used, 30 km h^{-1}, no difference was found between species (X^2 = 12.140; d.f. = 7; P = 0.096). The probability of falling into the water (Figure 3) was lower at 10 km h^{-1} with a rate of 12% for *P. acuta*, and 32% for *P. clarkii*. For a 20 km h^{-1} velocity, the probability for both species doubled, with a value of 40% for *P. acuta* and 70% for *P. clarkii*. For the highest velocity used, 30 km h^{-1}, the probability of falling into the receiving water body was near 100%, with a probability of 79% for *P. acuta* and 96% for *P. clarkii*.

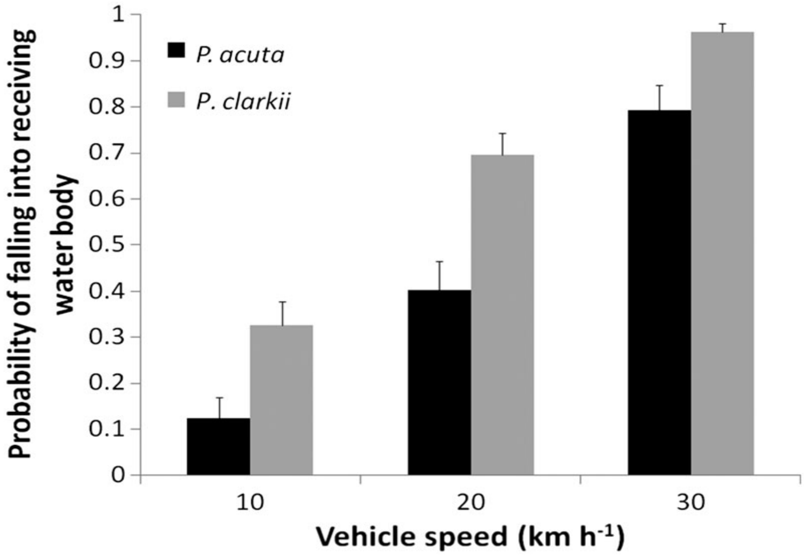

Figure 3. Probability of individuals (± SE) of *Physella acuta* and *Procambarus clarkii* falling into a receiving water body at different vehicle velocities (n = 60).

DISCUSSION

This study shows that recently-hatched crayfish (*P. clarkii*) and aquatic snails (*P. acuta*), can be transported successfully by off-road vehicles for long distances. The dispersal by this type of vector can have a high probability of success, since both species have a great capacity to survive out of water. In addition, the mud and its humidity attenuates desiccation, promoting the extension of survival time. In fact, recently-hatched *P. clarkii* can survive out of water more than 3 h at 24 ºC and 35% relative humidity, and these values correspond to a summer situation in the Iberian Peninsula (Banha et al., 2012). So, theoretically, considering this survival time, it may be expected that *P. clarkii* can be transported by motor vehicles up to distances of 270 km if the vehicle speed is 90 km h^{-1}. This theoretical distance corresponds to more than double the mean survival distance found in the study, but the calculations excluded the possibility that crayfish will fall off the vehicle as well as the antagonistic effects of the wind and mud on survival. *Physella acuta*, which is a pulmonate gastropod, apparently had a higher capacity to survive transport, since pulmonate gastropods can survive desiccation for several months (Barbosa and Barbosa, 1959; Cridland, 1967; Véra et al., 1994). Although no explicit desiccation survival data are available for this species, Bousset et al. (2004) found that *P. acuta* are able to survive drought periods, burying themselves in the mud during the summer. In theory, *P. acuta* have characteristics that allow them to be transported further than *P. clarkii*, but the results showed a mean transport distance only 10 km longer than for *P. clarkii*.

Another important aspect is that, at least in the Iberian Peninsula, both species are present at high densities in late autumn and early winter. Recruitment of *P. clarkii* is more intense in the autumn (Anastácio and Marques, 1995; Fidalgo et al., 2001; Anastácio et al., 2009; Sousa et al., 2013), during which time *P. acuta* is present also at peak density (González-Solís and Ruiz, 1996). In this period of the year, the low temperatures and high relative humidity are more favourable to long survival times out of water. In addition, this time of the year coincides with a period of intense activity for off-road vehicles near rice fields and adjacent water bodies. This happens because of the harvest of rice, the hunting season (Rodrigues and Fabião, 1997), and, at least in Portugal, the off-road tours and competitions that are very frequent in October and November (FPTT (Federação Portuguesa de Todo o Terreno Turístico, trial e navegação 4x4), 2013).

For high dispersal success by motor vehicles, Waterkeyn et al. (2010b) stated that car tyres should frequently come in contact with water and therefore promote the release of propagules in a suitable environment. In this work, it was demonstrated that a high velocity enhances the fall of propagules and of the mud attached to motor vehicles. It was also found that, if significant precipitation occurs during the transport process, the mud and the organisms attached to the vehicle will be promptly released on the road, possibly far from a suitable water body. There were slight differences between the rates of fall of the study organisms, and it is possible that these differences are caused by an organism's movements in the mud. *Procambarus clarkii* fell more than *P. acuta*, and the former can make leg movements that allow for fast detachment from the mud, while the absence of movements by *P. acuta* probably promoted its permanence in the mud. This allows *P. acuta* to be transported over much longer distances. On the contrary, during the first step of dispersal, i.e. adherence to the vehicle, no differences between snail and crayfish were detected. This is in accordance with the statement made by Vanschoenwinkel et al. (2011) that attachment of mud is probably not a selective process and propagule properties may matter very little when these are embedded in a sticky matrix.

Mud also plays a crucial role in an aquatic organism's passive dispersal by animals by promoting the adhesion and maintenance of the propagules on the vectors. This process has been described in mud-wallowing mammals such as elephants and wild boars (Vanschoenwinkel et al., 2008b, 2011) and for waterbirds. Moreover, Van Leeuwen and Van der Velde (2012) showed that snails, when deliberately attached to duck bills with mud, could remain there for up to 8 h. In fact, waterbirds have long been considered a major long-distance disperser of aquatic organisms (Figuerola and Green, 2002) and were identified as a dispersal vector for the study species. Rees (1965) regularly observed *Physa* sp. on the migratory upland plover *Bartramia longicauda*, which carried 10–30 snails under their wings upon arrival in Louisiana. Likewise, Banha et al. (2012) found that *P. clarkii* can be dispersed by waterbirds too, namely by mallard ducks (*Anas platyrhynchos*). Studies addressing dispersal by waterbirds (Banha et al. (2012) for *P. clarkii*, Boag (1986) for three freshwater snails (*Lymnaea stagnalis*, *Stagnicola elodes*, *Helisoma trivolvis*), and Van Leeuwen and Van der Velde (2012) for another three snails (*Gyraulus albus*, *Anisus vortex*, and *Radix balthica*)) mostly show a higher rate of adhesion to birds, but shorter transport distances compared with the results for dispersal by off-road vehicles obtained in this paper. A recent review showed that anthropogenic vectors were able to transport snails and bivalves to distances 10 times greater than natural vectors (Kappes and Haase, 2012). Therefore, for the species studied, dispersal by off-road vehicles can be a more relevant dispersal mechanism. The fact that vehicles can travel at a higher speed could promote higher success rates and longer distances for dispersal. In addition, motor vehicles are considered a very important dispersal vector for invasive plant species in terrestrial environments. Their seeds are transported over long distances along the roads, and this process is considered a routine mechanism rather than an exception (Von Der Lippe and Kowarik, 2007). It is possible that the last sentence can be transposed for aquatic organisms. In spite of the low probabilities obtained in this study for a single off-road vehicle dispersal of *P. acuta* and *P. clarkii*, the regular and intense off-road vehicle traffic between different water bodies promotes the process to become a very likely mechanism of invasion.

An invasion of *P. clarkii* should need a strong flux of individuals, since only juvenile individuals were transported by a vehicle vector. It is necessary that at least two individuals of both sexes are transported, that they grow and survive until sexual maturation, they reproduce and that reproduction is successful in producing a new and viable cohort. In contrast, a successful *P. acuta* invasion is much more likely because only one individual is needed, since this species is capable of self-fertilization and cars can transport adults. Although snails from the *Physa* genus are preferential outcrossing simultaneous hermaphrodites (Wethington and Dillon, 1993, 1996), the European wild populations of these snails demonstrate higher rates of self-fertilization (Monsutti and Perrin, 1999), and this could explain their wide distribution.

This work added relevant information for 'biosecurity' purposes by showing the importance of off-road vehicle traffic as a pathway for invasive freshwater invertebrates. This additional human vector for these species may help to explain their wide distribution on various continents and their success as invasive species. In areas where these two species (or related ones) are still expanding, it will be important for government authorities or agriculture associations to consider these findings and put in place the necessary measures to limit the passage of vehicles in contact with water in contaminated areas, especially during the autumn and winter. Limiting the circulation of 'potentially contaminated' vehicles between invaded and non-invaded areas could help to prevent impacts on biodiversity and direct economic losses.

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Chapter 4 - Live bait capture and crayfish trapping as potential vectors for freshwater invasive fauna

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Live bait capture and crayfish trapping as potential vectors for freshwater invasive fauna

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Abstract

Fishing activities strongly contribute to biological invasions in freshwaters. The purpose of this study was to investigate the potential risks of live bait capture using dip nets and of crayfish trapping as vectors for invasive freshwater macrofauna dispersal. In the Tagus river basin (Portugal), where both activities are common, we evaluated the probability of capture and the electivity of the local aquatic macrofauna according to the method used. During the compulsory removal of the invasive species captured we also quantified fish desiccation survival capacities. We found, for both vectors, that the species exhibiting the highest probability of capture and the highest electivity were invasive, respectively, *Gambusia holbrooki* and *Crangonyx pseudogracilis* with the dip net, *Procambarus clarkii* and several invasive species with special relevance for *Ameiurus melas* with the crayfish trapping. Moreover, the desiccation survival capacities, of all invasive fishes analyzed, are compatible with long distance dispersal out of water, with special relevance to *G. holbrooki*. This study demonstrates that fishing activities contribute to long-distance dispersal of invasive fauna. Therefore, according to our findings, it is important to update the fishing regulation and simultaneously to raise fishermen awareness of this problem.

Keywords: Biological invasions; Human vectors; Dispersal; Fishing activities; Desiccation survival; Fishing methods.

Introduction

The introduction and spread of invasive species is currently considered one of the major drivers in biodiversity loss world-wide (Moyle and Light, 1996; Pimentel et al., 2000; Strayer, 2012).In freshwater environments, this problem plays a crucial role (Ricciardi and MacIsaac, 2000) since these habitats are one of the major biodiversity reservoirs (Costanza et al., 1997). Thus, since the eradication of invasive species is rarely achieved (Costanza et al., 1997; Moyle, 2001; Simberloff, 2001, 2003), the prevention of invasion by acting on vector management is decisive. Therefore, the knowledge of the dispersal mechanisms is very important (Bohonak and Jenkins, 2003; Crooks and Soulé, 1999). Intentional introduction of aquatic organisms is the most important dispersal process in vertebrates, while the majority of the introductions are accidental in invertebrates (García-Berthou et al., 2007).

Many economical and recreational activities such as sport fishing and professional crayfish trapping, are closely associated within land aquatic systems and act as possible vectors for invasive fauna. Angling is connected to biological invasions due to the deliberated introduction of sport fish (Dextrase and Mandrak, 2006), due to the use of live bait (Ludwig and Leitch, 1996) and potentially due to accidental transport by gear related to fishing activities, such as for example, dip nets for bait capture. Consequently, many species of fish, crayfish, and of other invertebrates have become established in aquatic and terrestrial ecosystems out-side of their native ranges (Hobbs et al., 1989; Keller and Lodge, 2007; Picco and Collins, 2008). Crayfish traps are also potential vectors for accidental transport of invasive species since these are not thoroughly cleaned and are frequently moved among different locations. Both in the case of crayfish traps and of dip nets, the transport to another location involves tolerating exposure to air. Actually, this trait (i.e. desiccation survival capacity) is fundamental for passive overland dispersal of freshwater fauna (Banha et al., 2014; Figuerola and Green, 2002; Vanschoenwinkel et al., 2008).

The Sorraia River is the major Portuguese tributary of the Tagus river basin, occupying a large part of Central Portugal. Fishermen activities are very important in this area with numerous national and international competitions (FPPD, 2013). One of the most appreciated sports fishing species, is the non-native Large-mouth bass (*Micropterus salmoides*, Lacepède) (Godinho and Castro,1996). *M. salmoides* is traditionally fished using the native stone loach (*Cobitis paludica*, DeBuen) as live bait (Collares-Pereira et al., 2000). Although the Portuguese law has forbidden the capture and use of stone loach as bait in 2010 by the Portaria no. 624/2010, this technique is still used due to its efficacy for bass capture and low authorities' control. The capture of this bait involves the use of small-mesh nets (Collares-Pereira et al., 2000), a bucket with water and frequently vegetation to keep fish in good conditions. Consequently several invasive species can also be inadvertently transported by this process, both in the buckets and by the small-mesh dip nets while browsing for bait. Another fishermen activity that occurs frequently in this basin is red swamp crayfish (*Procambarus clarkii*) trapping. In fact, in the year 2000, more than 88 t of this species were captured in the Tagus river basin (Rodrigo et al., 2006). The capture of this species is legal, but may result in the transport of this or other invasive species to other locations. Since it is very difficult to obtain field data on illegal activities, such as intentional transport of non-native species or accidental transport on bait buckets, an indirect approach to assess the non-native aquatic fauna dispersal potential of these vectors was adopted. In this context, this work studies the electivity and the rate of capture of each species by crayfish trapping and by stone loach dip net capture. Due to its importance for the success of accidental transport, the desiccation survival capacity of the non-native fauna captured was also assessed.

Materials and methods

Data were collected in the winter and summer of 2013, using similar crayfish traps and dip nets to the ones used by local fishermen. During dip net searches for live bait and during crayfish trapping it is very common to capture other species and these were quantified. Additionally, in this study, none of the invasive species captured was released back to water, fulfilling the Portuguese law (decreto-lei no. 565/99 de 21 de Dezembro) and ICNF (Instituto de Conservação da Natureza e da Florestas) dispositions. Indeed, we obtained data concerning the desiccation resistance of the invasive fish species. The desiccation resistance of other detected invasive species, namely crustaceans (*P. clarkii* and *Crangonyx pseudogracilis*), is already available from previous studies involving the authors of this paper (Banha and Anastácio, 2014; Rachalewski et al., 2013). We selected 4 locations in the Sorraia River and tributaries (Tagus river basin) because these places are commonly visited by fishermen using both techniques (Fig. 1), but also because of its accessibility and permanence of water flow during all year. A quadrangular dip net was used, with a 1 mm mesh, 60 cm × 40 cm frame and a 120 cm cable. In each location 8 one meter long drags were performed, to quantify organisms from all different micro-habitats present. Eight galvanized steel wire crayfish cylindrical traps (1 cm mesh; 42 cm long; two 5 cm openings; 23 cm diameter) were used and placed in 8 different locations. Traps were baited with 50 g of sardines (*Sardina pilchardus*) immediately before dusk and checked the next morning. The non-native fish fauna captured by these two techniques was measured and the time to death was registered since according to the Portuguese legislation it is forbidden to return them to the water. We did not quantify the loss of invasive fauna during the transfer of live bait to the bait bucket or during cleaning of the traps or of the dip nets since this is highly dependent on each fisherman options and skills. Fishes were considered dead if no movement or reaction to manipulation was present. The relative humidity and air temperature were measured using a thermo-hygrometer and water temperature was measured using a multiparameter probe (Multiline-WTW). Wind direction and velocity were taken from the nearest meteorological station (Geophysical Center, University of Évora). To obtain the species' densities in each location, fish fauna was electro fished in 3 areas of 5 m^2, using a CPUE of 5 min. For invertebrates we used 3 areas of 1 m^2 isolated by mesh (1 mm), thoroughly sampled using a dip net until no more fauna was found, using the catch-success method (Leslie and Davis, 1939). Densities were estimated in both seasons (winter and summer). Statistical analysis Ivley's electivity index (Ei; Ivlev, 1961) was used to determine the vector preference for each species caught:

$$Ei = \frac{r_i - p_i}{r_{i+} p_i}$$

where r_i is the proportion of organism species $_i$ in the catch composition, and p_i is the proportion of the same species in the environment. E_i ranges between −1 and +1. Positive values indicate selectivity of the fishing gear, with a species overrepresented in the catch composition in relation to its availability in the environment. Negative values signify avoidance, with a species under represented in the catch composition in relation to its availability (Lechowicz, 1982).For each species in each sampling station, the probability of capture by a type of fishing gear is the number of captured individuals of that species divided by the number of individuals of all species captured. Confidence intervals (95%) for the Ivlev's index values were calculated according to Strauss (1979). Ivlev's index values were considered significantly different from zero ($p < 0.05$) if its confidence interval did not include zero. The same protocol was applied to the probability of capture for each gear and season. A Kaplan–Meier analysis was used to calculate the mean survival time for the captured non-native fish species. Except for *G. holbrooki*, we captured few individuals of the majority of fish species and therefore the latter were not subjected to further analyses. An ANCOVA was performed in order to analyze the effects of the covariate *G. holbrooki* total length (mm) and the factor season (winter and summer) on the desiccation survival time of *G. holbrooki*. Before applying this analysis, total length and time survival data were transformed ($\sqrt{x}$), in order to meet the assumptions of homogeneity of variances and normality. We also confirmed the homogeneity of the regression slopes before running the analysis. All statistical analyses were performed using PASW version 18.

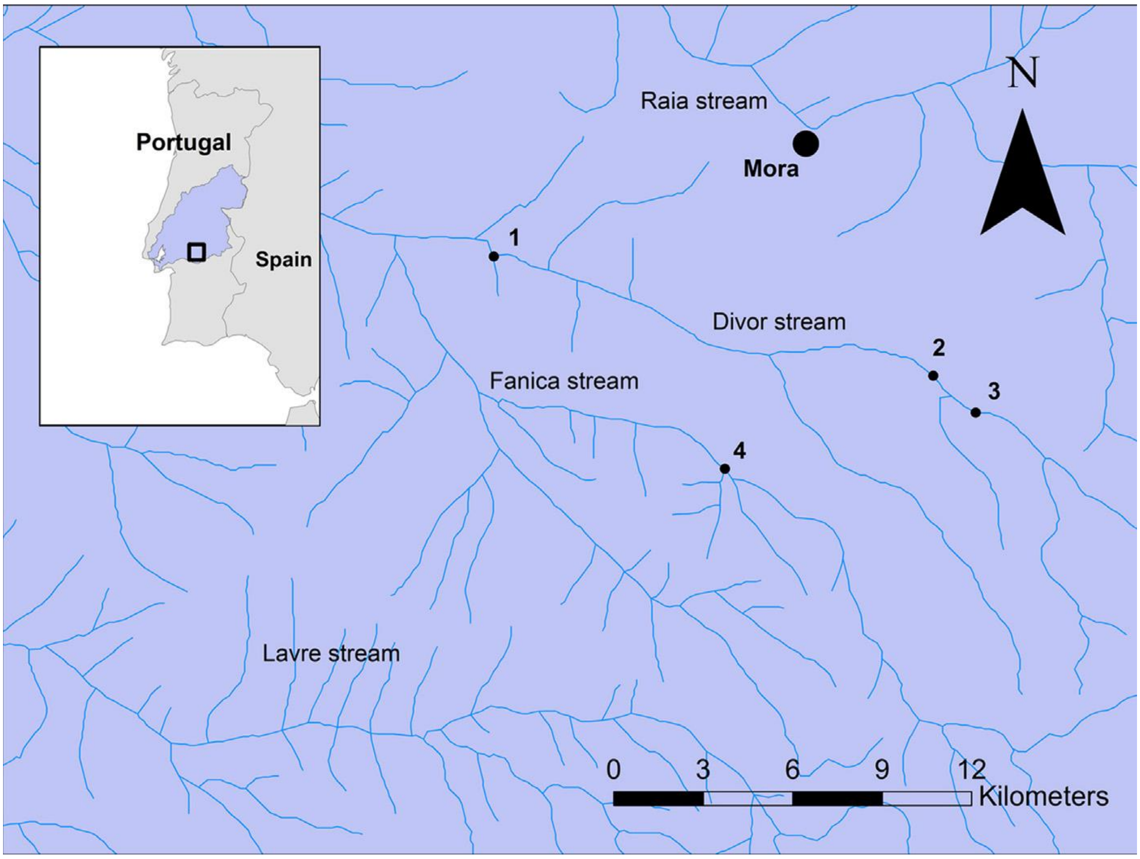

Fig. 1. Locations sampled in the Sorraia River (Targus river basin). Coordinates (WGS 1984 Datum) – location 1: 38.90226111 N; −8.260763889 W; location 2: 38.88228889;−8.176708333; location 3: 38.88213333; −8.167775; location 4: 38.841378; −8.199188889.

Results

In this study, 540 faunal organisms were captured, 64% of which were non-native. The total number of specimens captured slightly increased from winter to summer, from 34 to 43 specimens for crayfish trap, and 210 to 253 for the dip net. In fact, the faunal composition captured by each gear was different between seasons (Dipnet: X^2= 125.320; df = 12; sig. < 0.001; Crayfish trap: X^2 = 14.163,df. = 5, sig. = 0.015). The dip net allowed the capture of 13 different taxa, from which 7 were native. Nevertheless, the crayfish trap only allowed the capture of 7 taxa, with only 2 natives. So, the two types of fishermen vectors tested allowed the capture of

9 invasive species in the study area, namely 1 freshwater gastropod (*Physa acuta*), 1 amphipod (*C. pseudogracilis*), 1 crayfish (*P. clarkii*) and 5 fish species (*Alburnus alburnus*, *Ameiurus melas*, *G. holbrooki*, *Gobio lozanoi*, *Lepomis gibbosus*). The most abundant species found was the invasive amphipod *C. pseudogracilis* with a mean density of 12 individuals per square meter (Table 1) and its density was much higher in winter than in the summer. This species was one of the most captured by the dip net, having high values of probability (Fig. 2) and electivity in the summer (Fig. 3). Not surprisingly, due to its small size, no individual of this species was captured with crayfish traps and therefore, its electivity was −1 (Fig. 3). Although the dip net method is used by fishermen to capture *C. paludica* we obtained a very low capture rate. Actually, the crayfish trap had a higher probability of capture for this species. The second most abundant invasive species found was *G. holbrooki* with a mean density of 9 individuals per square meter, in both seasons. This species was more likely to be captured with a dip net, than with the crayfish trap. As expected, crayfish traps were very effective for *P. clarkii*, with a high probability of capture and a high electivity index (Fig. 3). Crayfish traps also presented high electivity for non-native bullhead catfish, *A. melas*, and captured other 5 fish species. This vector only captured two macroinvertebrate species, the abovementioned red swamp crayfish and the river shrimp *Athyaephyra desmarestii*, the most abundant native species found in the study area.

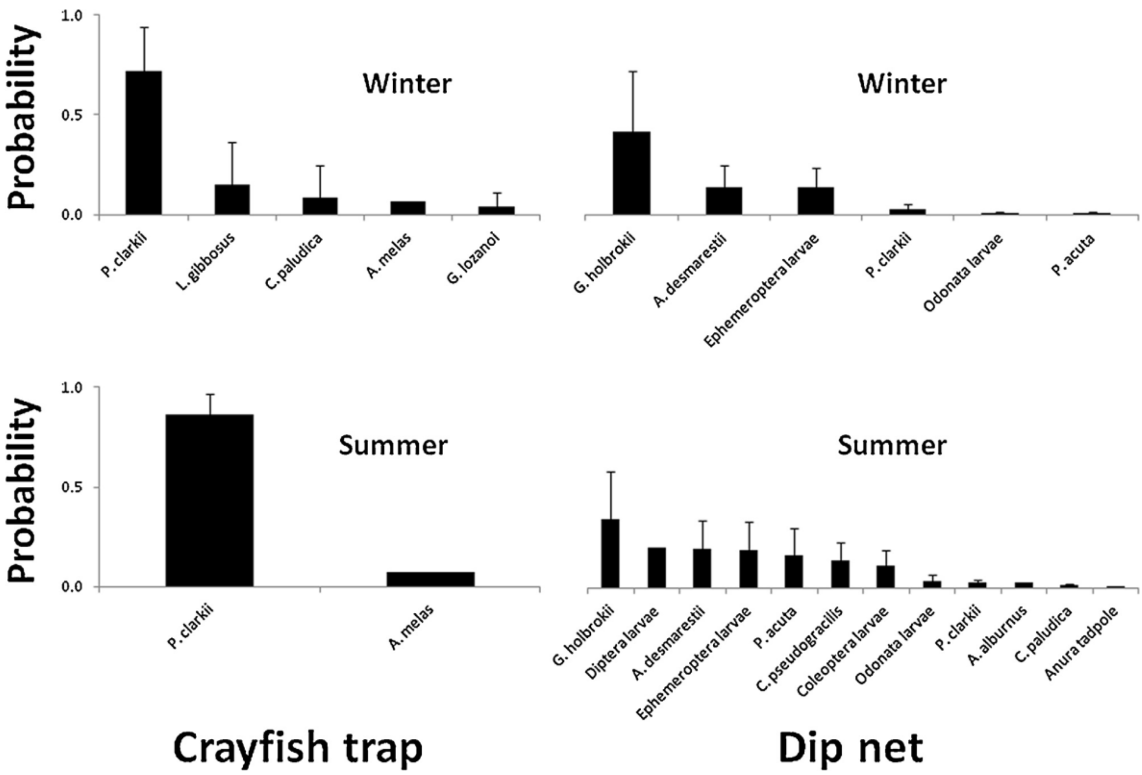

Fig. 2. Probability of capture (%) for each vector in the summer and winter. Only the species with a probability different from zero are displayed (zero not included in 95% confidence interval). Error bars correspond to 95% confidence interval.

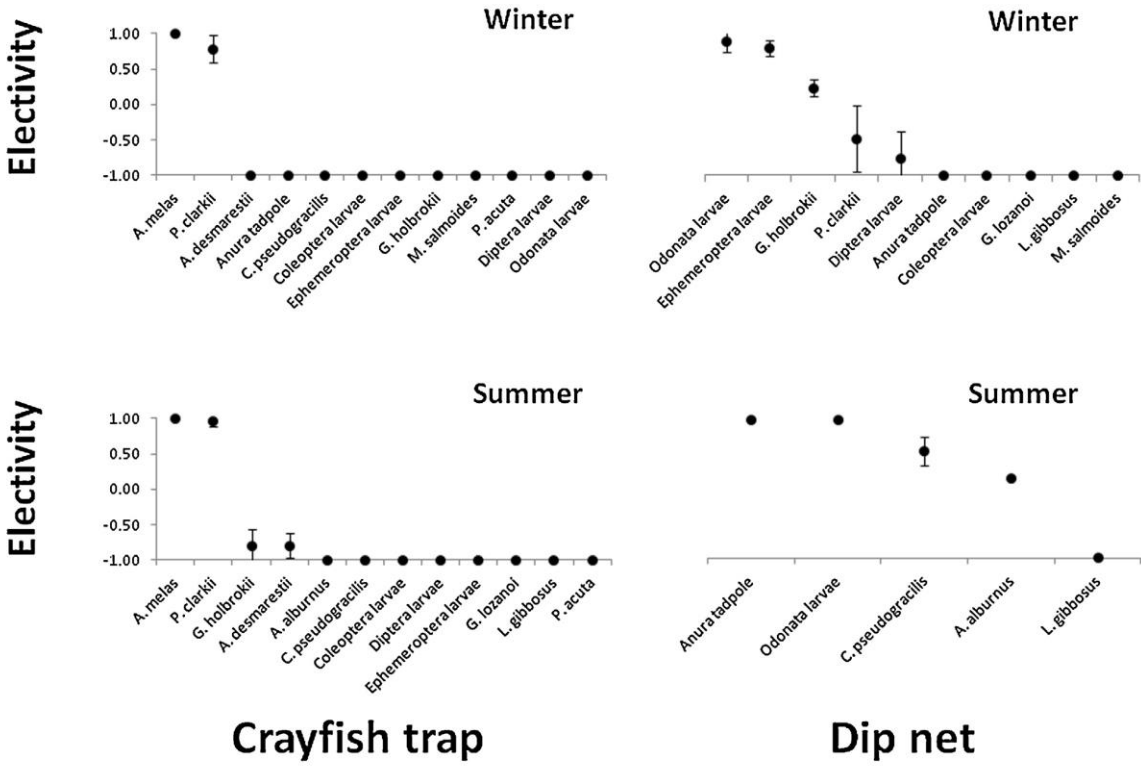

Fig. 3. Electivity for each vector in summer and winter. Only the species with a probability different from zero are displayed (zero not included in 95% confidence interval). Error bars correspond to 95% confidence interval.

Table 1

Mean density in each season for the aquatic organisms found in the study area.

		Mean density (individuals m^{-2})		
		Winter	Summer	Total Mean
Crustacea				
	Athyaephyra desmarestii	6.00	17.33	11.67
	Crangonyx pseudogracilis	23.14	1.00	12.07
	Procambarus clarkii	2.92	1.50	2.21
Actinopterygii				
	Alburnus alburnus	-	0.73	0.73
	Ameiurus melas	-	-	-
	Cobitis paludica	0.13	0.18	0.16
	Gambusia holbrooki	10.22	9.25	9.74
	Gobio lozanoi	0.33	1.03	0.68
	Lepomis gibbosus	0.20	0.28	0.24
	Micropterus salmoides	0.07	-	0.07
Insecta				
	Coleoptera larvae	2.01	16.50	9.25
	Ephemeroptera larvae	1.23	5.67	3.45
	Diptera larvae	5.30	2.50	3.90
	Odonata larvae	0.02	-	0.02
Mollusca				
	Physa acuta	1.22	7.50	4.36
Amphibia				
	Anura tadpoles	0.33	-	0.33

Table 2. Estimated mean fish desiccation survival time (in minutes) given by a Kaplan-Meier analysis.

		Survival time (minutes)			Biometrics			Environmental conditions		
Organism	Season	Estimated mean	95% confidence interval	Standard Error	Total length (mm)	Weight (g)	n	Air temperature (ºC)	Relative Humidity (%)	Wind speed (m/s)
G. holbrooki	Summer	39.5	37.2 – 41.8	1.2	25.23 ± 3.81 S.D.	0.14 ± 0.05 S.D.	90	14.29 ± 2.97 SD	42.42 ± 11.05 S.D.	2.53 ± 0.29 S.D.
	Winter	31.8	27.6 - 36.0	2.2	22.39 ± 3.49 S.D.	0.09 ± 0.05 S.D.	33	34.45 ± 0.81 S.D.	19.06 ± 1.44 S.D.	1.50 ± 0.51 S.D.
G. lozanoi	Summer	11.1	9.6 - 12.6	0.8	24.11 ± 3.00 S.D.	0.13 ± 0.04 S.D.	9	30.7 ± 0.26 S.D.	28.44 ± 0.53 S.D.	1.37 ± 0.50 S.D.
L. gibbosus	Winter	22.2	20.2 – 24.3	1.0	43.58 ± 5.70 S.D.	1.46 ± 0.67 S.D.	8	16.00 ± 0.50 S.D.	49.0 ± 1.0 S.D.	2.7 ± 0.20 S.D.

Kaplan–Meier analysis showed that *G. lozanoi* presented a mean survival time of 11.1 min, in summer conditions. Yet, in winter conditions, L. *gibbosus* presented a mean survival time of 22.2 min (Table 2). For *G. holbrooki* the Kaplan–Meier analysis showed a mean desiccation survival time of 39.5 and 31.8 min, in summer and winter conditions, respectively.

ANCOVA showed that total length explains the *G. holbrooki* desiccation survival time (Table 3). In fact, the survival time increases with the fish size (Table 2), but the season had no effect. There was also no interaction between season and total length (Table 3).

Table 3 *G. holbrooki* desiccation time analyzed using an ANCOVA (R^2 = 0.608; Adjusted R^2 = 0.594). Season (winter, summer) was a factor and Total length of the fish was a co-variable.

	Type III Sum of squares	d.f.	Mean square	F	Sig.
Correct model	43.598	3	14.533	42.990	<0.001
Intercept	3.225	1	3.225	9.540	0.003
Season	0.806	1	0.806	2.385	0.126
Total length	31.063	1	31.063	91.889	<0.001
Total length*Season	0.700	1	0.700	2.072	0.154
Error	28.058	83	0.338		
Total	3238.000	87			
Corrected Total	71.656	86			

Discussion

This work showed that both types of fishing methods studied can act as vectors for invasive aquatic fauna, being efficient in non-native species capture. The dip net used by fishermen to capture live bait (*C. paludica*) presented the highest risk for invasive fauna dispersal, since this vector captured more invasive species. This vector was not very efficient to capture its target species and had a high electivity for invasive species such as the amphipod *C. pseudogracilis*, the fish *G. holbrooki* and the crayfish *P. clarkii* (juveniles), which could be transported accidentally. Additionally, the process involves the transport of live bait in a bucket with water and enhances the probability of successful transport. The high number and small size of these nuisance organisms captured compared to the number of *C. paludica* captured makes it very difficult for fishermen to reject them and to transport only *C. paludica*. Moreover, the live bait used is normally captured in streams or rivers and used in artificial lakes where the Largemouth bass (*M. salmoides*) is more abundant (Almaça, 1995), ensuring a long distance transport. Actually, anglers which use fish live-bait are prone to discard excess fish either into the waterway where they are fishing or into local dam sand ponds, to provide bait for subsequent fishing trips (Lintermans, 2004). Studies in North America show that bait-bucket transfer is one major vector for fish transfer outside their normal range (Litvak and Mandrak, 1993; Ludwig and Leitch, 1996). Moreover, accidental transport may also occur in the dip nets, during fishermen movements along a river, or between rivers, when searching for *C. paludica*, since the species is not very abundant. In this case, although desiccation is a limiting factor, the nuisance species mentioned above have a great capacity to survive out of water. Taking into account our results, if a fisherman uses a car (90 km/h limit velocity) between locations, *G. holbrooki* can be transported to 59 and 48 km distances, out of water in dip nets, in summer and winter conditions, respectively, with a 50% chance of survival. Yet, *C. pseudogracilis*, considering its desiccation survival capacities obtained from the work of Rachalewski et al. (2013), can be transported in this way for 50 km. Actually, this is the distance between our study area and the location of the first record of the species (Grabowskiet al., 2012). So, the use of dip nets can be one explanation for the presence of this species in the study area and, comparing our findings with Rachalewski et al. (2013), may be a more important vector than waterbirds. Dip nets may also contribute for recently-hatched crayfish long-distance dispersal, since they can survive more than 3 h out of water (Banha et al., 2012) which would allow for transport distances up to 270 km. The accidental transport of large individuals or large species seems unlikely for dip nets or crayfish traps. However, adult *P. clarkii* and the majority of fish species, may be transported intentionally in bait buckets and this has been previously described (Hobbs et al., 1989; Keller and Lodge, 2007; Picco and Collins, 2008). Actually, our results indicate that transport without water may also be successful, facilitating the escape from the detection by the authorities. This could be an important process in the case of *P. clarkii* since the adult individuals can survive in these conditions for more than 15 h (Banha and Anastácio, 2014).

Our work showed that larger individuals of *G. holbrooki*, mostly mature females, were able to survive more time out of water. This is very important for the invasion success because one pregnant female may establish a population in a new place. Actually, this species is viviparous and the mean fecundity rises with female size, reaching 131 fry per female (Pen and Potter, 1991). The desiccation survival capacities of *G. holbrooki* seems to be higher than of *L. gibbosus* and *G. lozanoi*. Additionally *G. holbrooki* has a large environmental plasticity and is considered an extremely tolerant fish, capable of living in water quality that excludes most other fishes (Marchetti et al., 2004). Consequently, this species can easily be transported between places by people, inadvertently out of water (small size individuals), or intentionally in containers at great densities (larger individuals), without a high risk of mortality, contrary to other species.

Crayfish traps, showed a high efficiency translated by the high probability and electivity for its target species, *P. clarkii*, contrary to the dip net for *C. paludica*. The high level of efficiency, in part, results from enhancement due to its relevance for the crayfish production industry

(Kutka et al., 1992; Rach and Bills, 1987; Romaire and Osorio, 1989; Stuecheli, 1991). Based on our results, this vector seems of low risk for the dispersion of small macrofauna. The absence of small size organisms could be explained by the large mesh size. So, taking the results into account, we think that this vector may be only important for intentional dispersal of large freshwater fauna, particularly *P. clarkii* and non-native fish species. Pot gear is most efficient in the capture of bottom-dwelling species, seeking food or shelter (Everhart and Youngs, 1981), thence the higher electivity value found for the *A. melas*, a bottom fish species. Additionally, North American catfishes are commercially harvested with pot gear (Perry and Williams, 1987). Another species captured by crayfish traps was the pumpkinseed sunfish *L. gibbosus*. This was not strange since trap nets are efficiently used to capture sunfishes, being this method more efficient than gill nets (Hubert et al., 2012; Miranda et al., 1996).

Considering our findings, both vectors studied had implications on aquatic fauna dispersal, both native and invasive, with special relevance for *C. pseudogracilis* and *G. holbrooki* dispersal. Thus, to prevent the spread of invasive species, management efforts must aim to control human vectors of dispersal (Ruiz and Carlton, 2003). In this way, it is important to implement a program of invasive species awareness and aquatic fauna conservation among sport and professional freshwater fishermen. Also, it is important to extend legislation to totally abolish the use of live bait and enhance the fishing authorities' control.

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Chapter 5 - Angling web forums as an additional tool for detection of new fish introductions: the first record of *Perca fluviatilis* in continental Portugal

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Angling web forums as an additional tool for detection of new fish introductions: the first record of *Perca fluviatilis* in continental Portugal

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ABSTRACT

This work reports for the first time the presence of a non-native fish, the European Perch (*Perca fluviatilis*), in continental Portugal. The presence of this species was first reported on an angling web forum and its occurrence was scientifically confirmed later in a small reservoir of the Tagus river basin, located in the central region of Portugal. The importance of the angling web forums as a useful tool to help detection of non-native fish species introductions and their potential for education on biological invasions is discussed.

Key-words: Biological invasions, angling, freshwater introductions, invasive fish.

The extremely rich endemic freshwater ichthyofauna of the Iberian Peninsula is facing a growing risk mainly due to habitat destruction and the introduction of non-native species. At least two-thirds of the freshwater and migratory fish species in Portugal and Spain are threatened, and in many river segments and lakes, non-native species are now dominant both in number and biomass. In the continental Portuguese freshwaters, eighteen non-native species are currently established, representing from 25% to 30% of the total fish community in several basins, mainly in the southern region (Ribeiro et al., 2009; Ilhéu, unpublished data). In Spain, twenty-six non-native fish species are currently established in freshwater ecosystems, representing at least 30% of the current freshwater fish fauna list (Elvira and Almodóvar, 2001; Leunda, 2010). The main routes or pathways for fish invasions in the Iberian Peninsula are well identified, being the entryway, the French border, with an invasion route from east to west, from Spain to Portugal. Most of these introductions occurred during the late 20th century and many of them were related to angling, including illegal stocking of target species and bait/"forage" species release.

The European perch, *Perca fluviatilis* (Linnaeus, 1758), is a medium-sized fish from the Percidae family. It is native to Europe and Siberia as far as the Kolyma River, being naturally absent from the Iberian Peninsula, Southern Italy and parts of the Balkan Peninsula. This species has a great capacity for adaptation to different environmental conditions and a high fecundity, being successfully introduced in several regions of the globe, including Oceania and Africa. In the Iberian Peninsula this species was introduced due to its sport value in the early 1970s, its present distribution being mainly restricted to the vicinity of the French border, in the Catalonian river basins, including the Ebro River. This species has been successfully introduced into the Azores Islands (Ribeiro et al., 2009), but no previous report of the species occurrence has been made for Continental Portugal. This work aims to report the first occurrence of *P. fluviatilis* in inland Portuguese waters. Additionally, the importance of angling web forums as a useful tool to help detection of non-native fish species introductions and their potential for education on biological invasions is discussed.

A web search on the most visited and oldest Portuguese angling forum (http://www.portugalpesca.com, online since 2005; 5728 members; 261985 posts, accessed 18 August, 2013) was performed using the common name (in Portuguese) of non-native species present in Spain, but absent in Portugal, as keywords. After the analysis of the forum posts, including locations, photos and descriptions of the fish species, the report of *P. fluviatilis* catches (2 topics, 7 posts, 3 photos) was taken as potentially valid and field sampling was performed later on. The presence of *P. fluviatilis* in a small reservoir (Vale Longo; area = 3 ha) used for water supply by a conglomerate industry near Proença-a-Nova (Figure 1) was reported in the web forum from 2013. Two field surveys were conducted in this reservoir in February 2014. In the first one we assessed the reservoir conditions to select the fishing method, concluding the inefficiency of electrofishing due to the very deep (mean water depth 12 m) and clear water, with low conductivity (value 103 $\mu s \cdot cm^{-1}$). In the second one, a fishing rod was used with a small artificial lure technique. The session took 4 h, before sunset and a mature male (sexually active) *P. fluviatilis* was captured. No other individuals from this or from other species were captured, but 2 other small *P. fluviatilis* individuals (< 10 cm) were seen near the banks. The species identification was confirmed following Kottelat and Freyhof (2007) based on meristic features. The captured fish measured 17.2 cm standard length and presented 46.1 g of total weight. The specimen was transported alive in a bucket with water to the laboratory and preserved in 70% ethanol for future genetic analysis. During the field work, personal communications from three local fishermen revealed that this species is currently captured by anglers and its presence at this location dates from six to seven years ago. The gap between the date reported by the local fishermen (2007–2008) and the first post in the forum (2013) could be related to the reservoir's small size, low accessibility and number of fish species (Banha, unpublished data) and therefore its low attractiveness for anglers. The introduction of this species into continental Portugal nearly closes the list of potential predator fishes introduced primarily into Spain. We hypothesized that the long transport distance involved (more than 500 km) between this Portuguese small reservoir and the nearest well-established population in Spain (in the Ebro river basin), which may have functioned as a donor region, may be associated with illegal transport of *P. fluviatilis* specimens by recreational fishermen. The Ebro basin has a great influx of anglers from all over Europe (Binimelis et al., 2007). As an example, 11 international largemouth bass boat fishing competitions have occurred in the Mequinenza reservoir (Ebro basin) since 1992 and Portuguese teams have always been present in this competition, with 12 participants per year (www.caspebass.com). Thus, *P. fluviatilis* (as well as other non-native species) can easily be transported alive in the livewell of modern fishing boats and this would be a likely process of transport. A possible reason for the introduction of *P. fluviatilis* into this particular reservoir may be related to the absence of other fish predator species in this water body (Banha, pers. observation), namely the non-native species *Micropterus salmoides*.

Since *P. fluviatilis* is apparently confined to this small reservoir, the Portuguese authorities (ICNF) were contacted so that an eradication plan could be started promptly. We propose lowering the water level of the reservoir, if possible reusing the water downstream, followed by selective electrofishing, with total removal of the individuals of this species and the rescue of native fishes. Additionally, a monitoring plan should be implemented to confirm the efficacy of the eradication plan during a 5–10-year period.

The first reported location of *P. fluviatilis* presence in Portugal is very close (less than 30 km) to the location of the first record of wels catfish (*Silurus glanis* L.) in the Portuguese Tagus river basin. The Tagus river basin in Portugal presents one of the highest richnesses of non-native predatory fishes, with the occurrence of zander (*Sander lucioperca* L.), pike (*Esox lucius* L.), largemouth black bass (*Micropterus salmoides* Lacépède), wels catfish (*Silurus glanis* L.) and now *P. fluviatilis*. The anglers' preference for non-native game fishing, the availability of fishing guide services in the area and the influxes of fishermen may increase the introduction rate of non-native species in inland waters.

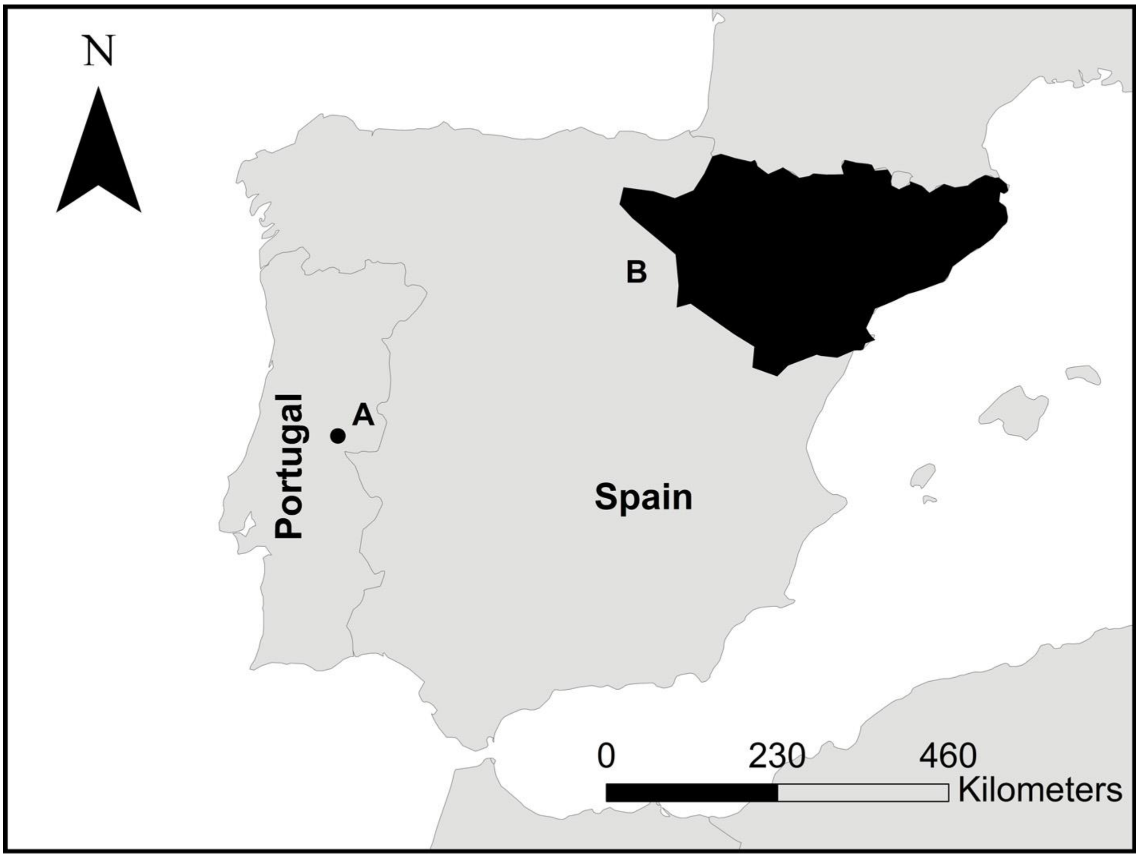

Figure 1

Perca fluviatilis distribution in the Iberian Peninsula. A- First record in Portugal, Vale longo reservoir in the Tagus river basin, reported in this work; B – invaded area in Spain in the Ebro and Catalonian river basins.

The anglers' preference for non-native fish species may be related to the absence of native predatory or game fish species in the majority of the Portuguese river basins, particularly in the central and southern regions (Matono et al., 2012). In fact, native game fish species, namely the brown trout (*Salmo trutta* L.) and the Atlantic salmon (*Salmo salar* L.), are present mainly in the Northwest and in the mountain rivers of the Northern-Central region of Portugal (Cabral et al., 2005; Matono et al., 2012). Additionally, since the introduction of the largemouth black bass (*Micropterus salmoides*) in the 1950s, by the National Authority for Inland Fisheries (Ribeiro et al., 2009), the importance and popularity of predatory fish, as well as the number of recreational anglers, has increased considerably in the last few decades (Santos et al., 2006). Portuguese law (Decreto-Lei no. 565/99) forbids the introduction of non-native species; however, many illegal fish introductions have been taking place in the country, due to the lack of fishermen's awareness of biological invasion problems and inefficient control by the national authorities. Regrettably, Portuguese fishing law protects some of the oldest invasive species introduced (e.g. largemouth black bass) by applying minimum capture size and non-fishing periods (see http://www.icnf.pt/portal/pesca/pdesportiva/calen-min). Fortunately, for the most recently introduced fish species these restrictions were not applied. In conclusion, anglers and environmental managers have conflicting interests. Non-native fish species recurrently introduced by anglers constitute a major concern and a great threat to native fauna through predation, competition, hybridization and transmission of new diseases to native fauna (see Leunda, 2010; EEA, 2012). For example, one of the most valued game fish species in Portugal, the non-native largemouth bass *Micropterus salmoides*, clearly affects the native fish community's structure (e.g. Godinho and Ferreira, 1998).

In Portugal, there are currently more than 11 active web angling forums dedicated to freshwater fishing activities, corresponding to more than 357000 users and 1700000 posts. In these forums there is information about fish species abundance, places of capture, methods of capture, baits, common names used, the sport and food value of each species, photos, methods used for illegal capture and even places suggested for future introductions. We found several fishermen's posts wishing for the spread of *P. fluviatilis* (4 posts), *Rutilus rutilus* (5 posts) and the introduction of other non-native species, namely *Abramis* sp. (4 posts). The information and reports posted on the angling forums are not rigorous but can be extremely useful for planning field sampling. New fish species reports in these forums should be considered reliable if the posts are numerous and from multiple authors and if these posts provide precise locations and good quality photos of the specimens. However, even reliable posts should be subjected to *in situ* scientific validation. Therefore, it is important to establish a link between the forums' administrators and the scientific community, environmental managers and the related government agencies, allowing a rapid response to the detection of new fish species. This partnership could be even more important or vital as a tool for invasive species awareness and environmental education, since the information easily reaches a great number of target people, preventing future introductions.

The information accessed in angling forums was already essential for Ribeiro and Veríssimo (2014), who published the first record of other non-native game fish in Portugal, namely the roach, *Rutilus rutilus* (Linnaeus, 1758). In Portugal, there is no specific sampling network exclusively for non-native species detection. This information is collected by a government agency (ICNF) from several diffuse sources (e.g. scientists' reports, fishing competitions supervised by ICNF, and EU Water Framework Directive data). Therefore, angling forums may provide additional information, complementing other sources and improving monitoring and action plans in the field of aquatic invasions.

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Chapter 6 - The role of angler's perceptions and habits in biological invasions: an international assessment

This chapter is submitted to an international peer-reviewed journal:

Banha F., Diniz A. and Anastácio P.M. (under review) The role of angler's perceptions and habits in biological invasions: an international assessment.

The role of angler's perceptions and habits in biological invasions: an international assessment

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Abstract

1. Anglers are a recognized vector for invasive fauna, with both intentional and accidental introductions reported worldwide.
2. The purpose of this study was to investigate the Iberian freshwater angler's habits and perceptions related to biological invasions, using an international and bilingual survey in Spain and Portugal.
3. Our results showed that anglers have a great mobility, with no differences between countries in distances traveled to the fishing locations.
4. The majority of anglers fish during consecutive days and visit several places. Yet, angler's activity patterns throughout the year were not similar. For both countries, the preferred fish species were invasive and its introductions were reported more often than native species.
5. The categorical motivations chosen for introductions were country dependent. A low percentage of anglers use live bait, sometimes invasive species, and discharges of unused bait in the water are very frequent.
6. The majority of anglers have the perception that introductions have environmental impacts and that anglers have an active role in intentional introductions. However, only a minority is aware of the angler's role on accidental transport of invasive species.
7. Our findings on angler's behaviors and perceptions may be used to model invasion risks and also to improve governmental agencies monitoring and awareness programs.

Keywords: Angling; dispersal; freshwater; invasive species; sport fishing; survey; introductions.

Introduction

Freshwater ecosystems are one of the most endangered ecosystems in the world, presenting higher declines in biodiversity than the most affected terrestrial counterparts (Sala et al., 2000). The Iberian Peninsula freshwaters are no exception, being an example of conservation concern mostly because of the existence of a high number of endemic (Doadrio, 2001) threatened species (IUCN, 2013). These species are being affected by profound flow and channel morphology modifications. Additionally, water quality is being depleted by industrial waste and sewage effluents which favors the invasion of non-native species (Elvira, 1995). In fact, a large number of invasive species are already established in Iberian Peninsula (García-Berthou et al., 2007) and sport-fishing related activities are an important vector. Although the major non-native species stocking efforts have been suspended by governmental authorities, many fish species have been introduced illegally by anglers, since the last half of the 20th century (Elvira and Almodóvar, 2001). Not only "game fish species" have been introduced worldwide by this vector. Anglers' use of live bait contributes to the dispersal of other aquatic organisms (Banha and Anastácio, 2015; Drake and Mandrak, 2014; Nathan et al., 2015) such as fish, crayfish and other invertebrates (Ludwig Jr and Leitch, 1996). Other non-bait organisms and pathogens have also been accidentally introduced by water or packing material of live bait containers (Goodchild, 2000; Haska et al., 2012) and, in the Iberian Peninsula, one example is the zebra mussel (*Dreissena polymorpha*) (Binimelis et al., 2007).

For invasions management the knowledge of the dispersal mechanisms is very important, allowing the prevention of invasions by acting upon vectors (Bohonak and Jenkins, 2003; Crooks and Soulé, 1999). So it is essential to know freshwater angler's activities, like the use of live bait, movements, type of gear used, fish species preference and others that may affect accidental and intentional introductions. Additionally, the assessment of anglers' perceptions on introductions and its implications should be assessed to optimize future awareness campaigns. The use of surveys has been successfully used to assess anglers perceptions (Gozlan et al., 2013; Lindgren, 2006) and risk behavior (Keller et al., 2007; Kilian et al., 2012), constituting an adequate tool. Until now, this methodology has only been used at a regional or national scale. In this context, the aim of this work is the study of freshwater angler's habits and awareness of biological invasions in Portugal and Spain. Namely, we intend to verify the prevalence of anglers actions related to intentional and accidental introductions, identifying the groups and factors associated to both processes. Moreover, we will assess the anglers' perception of their actions and consequences of introductions. These two countries share many watersheds, as well as several native and non-native freshwater species. However, they present some differences regarding angling legislation and socioeconomic and cultural background. Therefore, it is also our goal to verify if there are differences in the prevalence of some risk behaviors, but also in perceptions regarding biological invasions.

Methods

Survey design and implementation

To achieve our goals, an internet survey was applied (see appendix and Table 1). Its design was based on Tuckman (2000) and Ghiglione & Matalon (2001). A pilot survey, was applied individually by one of the researchers, to 7 male anglers (22 to 65 years old). At the end, a short individual briefing took place, to discuss difficulties and suggestions about the survey. To obtain the Spanish version of our survey we applied the methodology described by The International Test Commission (ITC) guidelines (2010) and principles outlined in Hambleton & Patsula (1999). In the first step we asked three independent bilingual individuals of Spanish origin to translate the Portuguese survey version into Spanish language. In the second step, each Spanish version was given to one bilingual Portuguese, to back translate it to Portuguese. The paper authors met to elect the final Spanish survey version, following Stemler (2001, 2004). Each researcher played the role of an independent judge, evaluating the back translations. Each back translated question was compared with the original Portuguese version. For this purpose a score from 1 to 3 was used, in which 1 corresponds to a full match, 2 to a rough or approximate match, and 3 to a mismatch. Inter-rater consensus was assessed through Cohen's kappa (Cohen, 1960). One of the three back translations and its translated version were rejected by unanimity due to its imperfection. The evaluation of the other two back translations resulted in a kappa value of 65 and 61%, corresponding to a substantial strength of agreement between judges (Landis and Koch, 1977). The version with highest agreement was chosen to be the base of the final Spanish survey version. Note that in this version the questions without agreement between judges, where replaced by their respective counterpart in the other version, which presented total inter-rater agreement.

The survey was available on-line using the Google drive® tool for on-line surveys and actively advertised in 8 Portuguese and 7 Spanish angling forums, covering all different freshwater angling techniques. The use of angling forums allows reaching the majority of anglers population and has proven before to be a very useful tool to obtain information regarding biological invasions in freshwaters (Banha et al., 2015; Gozlan et al., 2013; Patoka et al., 2014) .

Survey contents

Portuguese and Spanish versions of the survey included 26 questions each (see appendix and Table 1). First, a brief introductory paragraph was presented, where the goal of the survey was mentioned, i.e. to obtain information about freshwater anglers habits. In this paragraph it was also mentioned that only the authors of this article had access to the responses and that the responses were confidential and anonymous. The survey started by asking for the personal characteristics of the responder, namely residence location, age, gender, education habilitations and job. These socioeconomic aspects were asked to assess its relations with angler's perceptions or habits related with biological invasions. Then, the questions were presented (see appendix and Table 1) sequentially. The responders were only given access to each question answering the previous one.

Survey analysis

Three educational levels were considered [level 1 (basic formation) ≤ 4th grade; level 2 (intermediate formation) = 5th to 12th grade; and level 3 (superior formation) > 12th grade]. Also, three levels were applied to angler's professional situation: level 1 = low (unskilled workers in commerce, services, agriculture, fishing, construction, industry and transports); level 2 = medium (salesmen, skilled workers in agriculture and fishing, technicians and administrative professionals); level 3 = high (upper management and specialists in intellectual and scientific professions) (Diniz et al., 2011).

The responses of the answers with a 10 options ordinal scale from "never used or done" till "always used or done" were converted into 0 or 1 corresponding to "not used or done" and "used or done", respectively. The purpose of the use of an *a priori* large scale and not a binomial one was to obtain the maximum number of valid responses. If a binomial option was applied, an infrequent practice could correspond to a false negative response.

Table 1 – Survey contents with question topics in English, response type and purpose of each question.

Question	Topic of the question	Question type	Response type	Purpose
1	Distance covered by anglers to their home's closest fishing place	Compulsory	Open, short space for writing the number of Km	Anglers' mobility and activity patterns. Distances involved in invasions promoted both by accidental and intentional transport by anglers, but also information about propagule pressure, throughout the year
2	Distance covered by anglers to their home's farthest fishing place			
3	Month with more fishing activity	Compulsory	Multiple choice, months of year	
4	Month with less fishing activity			
5	Number of fishing days per week during the season with more fishing sessions	Compulsory	Multiple choice, 10 classes' ordinal scale from "never" to "always".	
6	Number of fishing days per week during the season with less fishing sessions			
7	Frequency of fishing in rivers			
8	Frequency of fishing in lakes			
9	Frequency of fishing during consecutive days			
10	Frequency of visits to other countries to fish			
11	Frequency of fishing in multiple places during the same day			
12	Frequency of live bait use	Compulsory	Multiple choice, 10 classes' ordinal scale from "never" to "always".	Evaluate prevalence of risk behaviors, recognized by its potential for both accidental and intentional transport of invasive species
13	Name of the 3 animals most used as bait	Compulsory only for responders who did not answer "never"	Open, line for writing the names of animals	
14	Frequency of live bait release		Multiple choice, 10 classes' ordinal scale from "never" to "always".	
15	Frequency of capture of live bait in a different place from the one used for fishing			

Table 1 (continued) – Survey contents with question topics in English, response type and purpose of each question.

Question	Question content	Question type	Response type	Purpose
16	Frequency of keepnet use	Compulsory	Multiple choice, 10 classes' ordinal scale from "never" to "always"	Evaluate prevalence of risk behavior, recognized by its potential for both accidental transport of invasive species
17	Frequency of waders use			
18	Frequency of navigation devices (float tubes, kayaks and boats) use			
19	Perception of frequency of accidental transport on fishing gear	Compulsory	Multiple choice, 10 classes' ordinal scale from "never" to "always"	Evaluate which are the topics with less associated knowledge and which are the less informed social groups, to be able to implement awareness programs
20	Perception of intentional aquatic organism introduction by other anglers			
21	Name of the 3 preferred fish species for angling	Compulsory		Evaluate if anglers prefer fishing invasive or native species
22	Do you know anyone that introduced aquatic species in a waterbody?	Compulsory	Binominal choice between "yes" and "no"	Evaluate the perception of the frequency of introductions and of which species are most introduced
23	Name of the introduced species	Compulsory for responders that answered "yes" to the previous question	Open, 2 lines given to write the names of species	
24	Motivations for the introduction of species into a new waterbody	Compulsory	Open, 1 line given to write the motivation	Understand what promotes introductions in order to be able to suggest measures to minimize it
25	The introduction of a novel species into a waterbody presented environment impacts	Compulsory	Binominal choice between "yes" and "no"	Evaluate the anglers' invasion awareness and identify the groups with less knowledge
26	Which are the impacts	Compulsory for responders that answered "yes" to the previous question	Open, 3 lines given to write the impacts	

Statistical analyses were performed using PASW version 18 with the exception of circular data which was analyzed using Oriana version 4.01. To test differences between countries in the occurrence of each activity, a Chi-square test on a 3 way-contingency table was applied. The responses given (yes or no) were rows, country (Portugal or Spain) were columns and the questions were layers. The groups of topics included were – (1) Use of fishing gear: boats (boats, kayaks, float tube); waders (waders, boots); keepnet. (2) Travelling and angling: several places; other country; consecutive days. (3) Angler's perceptions of: intentional transport (introductions by other anglers); accidental transport (by themselves); if they know anyone (that introduced aquatic species), impacts (of introductions). Additionally, Two-sample Kolmogorov-Smirnov tests were applied to age composition differences between the groups giving affirmative or negative responses to the previous topics.

A Mann-Whitney *U* test was used to verify if there were differences between countries in: (1) angler's travel distance to the fishing places closest to their homes; (2) angler's travel distance to the fishing places farthest to their homes; (3) anglers activity per week during the maximum fishing activity period; (4) anglers activity per week during the minimum fishing activity period. A Chi-square test of uniformity (for circular data) was applied to data from each country to test if there was a homogenous distribution of the angler's activity throughout the year. The answers with "open" response, namely the "name of the live bait used" and "name of the 3 most preferred fish species" were analyzed and when possible the common names were converted into the scientific names and the frequency of occurrence was evaluated for each country.

For the last question, "which are the environmental consequences of introductions" the responses were individually evaluated by the 3 authors, for the explicit inclusion of all 3 topics, "impacts on: native species; ecosystems; ecosystems services provided to humans.", extracted from EEA (2012) technical report on invasive species. The classification followed a rank from 0 to 3, with 0 – corresponding to no response or a totally wrong answer; 1 – very incomplete answer with only 1 topic addressed, 2 – incomplete answer with 2 topics addressed and 3 - correct answer, with all 3 topics. The evaluation of the responses presented a kappa value of 86.6%, corresponding to an excellent strength of agreement between judges (Landis and Koch, 1977). For responses without agreement, an oral debate with all judges was performed until a total inter-rater agreement was reached.

A Mann-Whitney *U* test was applied to assess differences in ranks between countries. The results of the open question "Motivation of the introduction" were subjected to a similar analysis, but in this case the judges analyzed the responses individually and discussed until consensus defining 8 types or categories of response. Differences between countries were tested using a chi-square on a contingency table. The same statistical test was used for anglers preference for lakes or rivers, and also for the proportion of affirmative versus negative responses to "Do introductions have impacts?" and "Do you know anyone that introduced aquatic species?".

A non-metric Multidimensional Scaling (nMDS) was performed for each country's data. The variables were– (1) Use of fishing gear: Boats (boats, kayaks, float tube); waders (waders, boots); keepnet; live bait. (2) Travelling and angling: Several places; other country; consecutive days. (3) Angler's perceptions of: intentional transport (introductions by other anglers); accidental transport (by themselves); if they know anyone (that introduced aquatic species), impacts (of introductions). (4) Angler's characteristics: gender and educational level. The data was standardized to 0-1 values, since we used Euclidean distance as measurement of dissimilarity and it is sensitive to differences in the magnitudes or scales of the input variables (Milligan and Cooper, 1988). Complementarily, a hierarchical cluster analysis was performed on the same data and also using Euclidean distance as measure of dissimilarity. Clustering was made using Wald method, since it creates groups without increase of variance and heterogeneity (Ward Jr, 1963), being considered one of the best cluster methods (Ferreira and

Hitchcock, 2009), presenting the highest level of accuracy (Blashfield, 1976). The hierarchical cluster analyses allowed us to further assess the validity of the nMDSs and facilitated the visualization of its results (Clarke and Warwick, 2001).

Results

We obtained a total of 410 survey responses; from Portugal 259 answers (15 - 75 years old, median = 37). Most of the Portuguese anglers (71.0%) had middle school level, 27.4% had high school level and only 1.5% had basic school level. Concerning professional level, 45.9% of Portuguese anglers had lower level employments, followed by 35.1% in the medium and 18.9% in the high levels. The majority of anglers were men; and only 0.8% were women. Portuguese responders were distributed by region as: 5% Aveiro; 7.7% Beja; 6.9% Braga; 0.8% Bragança; 4.2% Castelo Branco; 7.7% Coimbra; 5% Évora; 1.2% Faro; 1.9% Guarda; 1.2% Leiria; 12.7% Lisboa; 5% Portalegre; 15.8% Porto; 10.8% Santarém; 3.5% Setúbal; 3.5% Viana do Castelo; 1.5% Vila Real; 5.4% Viseu. There were 151 Spanish responders (16 - 74 years old, median = 32). The proportion of Spanish anglers with middle and high school formation was 53.6% and 42.4% respectively, yet only 4.0% had basic school formation. Regarding the professional level, the majority of Spanish anglers had lower level employments (55.6%), 26.5% had medium level and 17.9% had high level employments. Like in Portugal, in Spain the majority of anglers were men and only 2.6% were women. The distribution of the Spanish responders was: 21.2% Andalucía; 2.6% Aragón; 1.3% País Vasco; 19.2% Cataluña; 8.6% Castilla-La-Mancha; 6.6% Castilla y León; 10.6% Extremadura; 2% Galicia; 0.7% La Rioja; 20.5% Comunidad de Madrid; 6% Comunidad Valenciana.

There were no significant differences in the distances that Portuguese and Spanish anglers travel to the fishing locations closest to their residences (Mann-Whitney $U = 18754.5$; $Z = -1.191$; $p = 0.489$; $N = 410$) nor in the distances of the fishing locations farthest to their residences (Mann-Whitney $U = 18754.5$; $Z = -0.692$; $p = 0.489$; $N = 410$). When anglers fish near home, more than 80% go to fishing spots closer than 40 km, being the mean distance 25.4 km (±40.4 S.D.). When anglers perform large fishing trips, more than 70% go to locations until 300 km, being the mean distance 251.3 km (± 234.1 S.D.) (Figure 1).

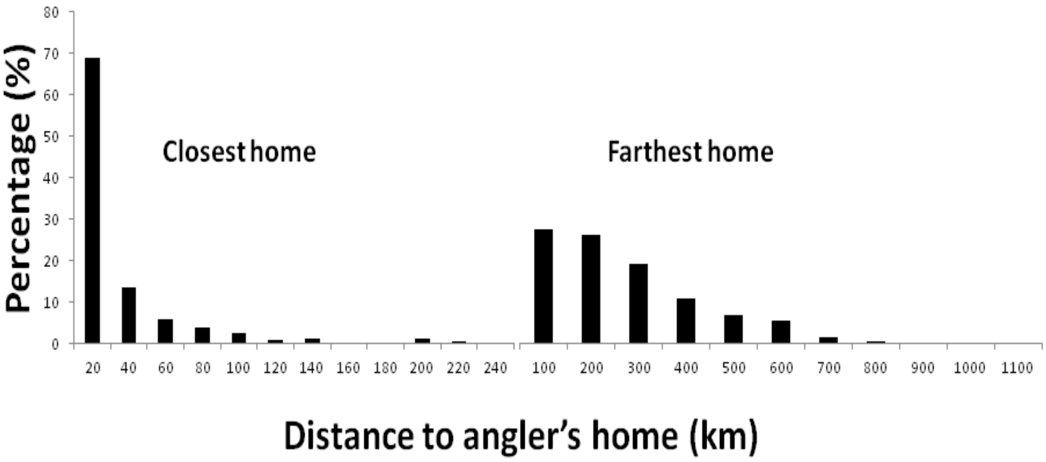

Figure 1- Relative frequencies of the reported distances to the fishing spots closest and farthest from home.

A Chi-square test of uniformity showed that fishing activity is not homogeneous throughout the year (Figure 2): Portugal – month with less activity; (χ^2 = 594.2; *df* = 11; $p <$ 0.001; n = 259) and month with most activity; (χ^2 = 261.3; *df* = 11; $p < 0.001$; n = 259); Spain – month with less activity (χ^2 = 104.3; *df* = 11; $p < 0.001$; n = 151) and month with most activity (χ^2 = 374.1; *df* = 11; $p < 0.001$; n = 151). In Spain the month with more angler activity is June (mean vector (μ) = 156.4°; length of mean vector (r) = 0.478; 95% Confidence Interval = 143.7-169.1°) and the month with less activity is January (mean vector (μ) = 3.0°; length of mean vector (r) = 0.667; 95% Confidence Interval = 354.8-11.3°). In Portugal, the month with less activity is also January (mean vector (μ) = 4.9°; length of mean vector (r) = 0.672; 95% Confidence Interval =

358.6-11.2°) but the month with most activity is July (mean vector (μ) = 180.2°; length of mean vector (r) = 0.645; 95% Confidence Interval = 173.5-186.8°) (Figure 2). A Chi-square test for circular data also showed monthly fishing activity differences between countries (Portugal, greatest activity month vs. Spain, greatest activity month – $\chi^2 = 51.9$; $df = 11$; $p < 0.001$; $N = 410$; Class width = 30°; Portugal, lower activity month vs. Spain, lower activity month – $\chi^2 = 41.1$; $df = 11$; $p < 0.001$; $N = 410$; Class width = 30°).

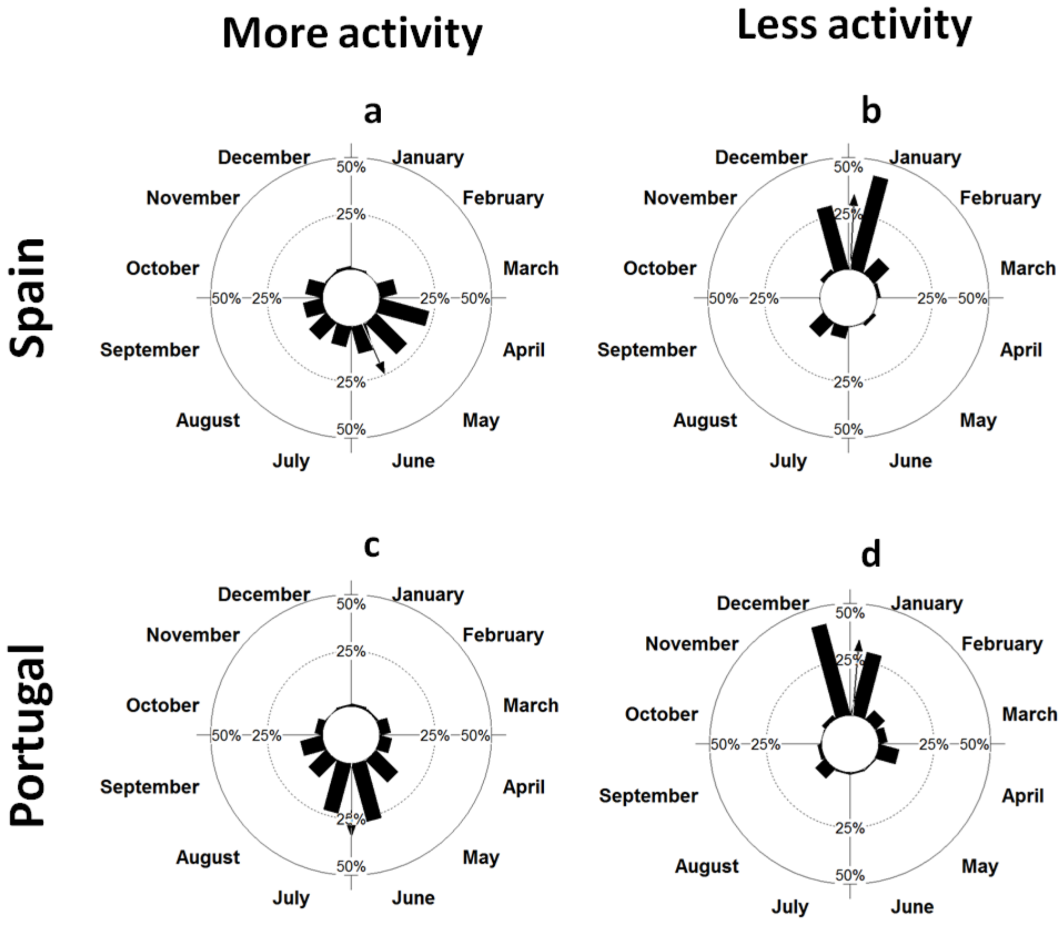

Figure 2 - Sports fishing activity throughout the year in each country. Relative frequency (black bars) and mean vector (arrow) are displayed. Different letters (a, b, c, d) mean different frequency distributions throughout the year (Chi-square test; p < 0.001). In all 4 cases the distribution was not uniform all year round (Chi-square test; p < 0.001).

There are no differences between Portuguese and Spanish anglers regarding the number of fishing days per week during the high activity period (Mann-Whitney U = 19073.0; Z = -0.429; p = 0.668; N = 410) and low activity season (Mann-Whitney U = 18916.0; Z = -0.617; p = 0.537; N = 410). During the high activity season the mean number of fishing days per week was 2.8 (± 1.6 S.D.) and 2.6 (± 1.4 S.D.) for Spain and Portugal, respectively. Yet for the low activity season, the number decreased for 0.6 (± 0.8 S.D.) days for Spain and 0.7 (± 1.0 S.D.) days for Portugal.

There are no differences in frequency of use of lakes and rivers by Portuguese (χ^2 = 0.000; df = 1; p = 0.986; n = 259) or Spanish anglers (χ^2 = 0.095; df = 1; p = 0.757; n = 15). Also, there are no differences between countries in the use of rivers (χ^2 = 0.230; df = 1; p = 0.632; N = 410) or lakes (χ^2 = 0.000; df = 1; p = 1.000; N = 410).

The proportion of anglers that know someone that introduced an aquatic species in a new waterbody (Figure 3) is higher for Portugal (35%) than in Spain (18%) (χ^2 = 12.495; df = 1; p < 0.001; N = 410). Both countries have similar proportions of anglers (approx. 90%) that think that introductions have impacts on the environment (Figure 3) (χ^2 = 0.005; df = 1; p = 0.946; N = 410). A similar percentage (approx. 95%) of anglers from both countries believe that other anglers practice introductions (χ^2 = 0.469; df = 1; p = 0.493; N = 410). Only a minority (approx. 30%) of Iberian anglers think that they accidentally transport aquatic organisms (χ^2 = 0.637; df = 1; p = 0.425; N = 410). Both in Portugal and Spain, anglers have similar mobility with the majority visiting several places during a fishing session (χ^2 = 0.430; df = 1; p = 0.512; N = 410) and fishing during consecutive days (χ^2 = 0.454; df = 1; p = 0.500; N = 410). Yet, in Spain, the number of anglers that visit other country to fish is small (30%), while half of the Portuguese anglers fish abroad (χ^2 = 10.230; df = 1; p = 0.001; N = 410). Regarding fishing gear, the use of a keep net was more frequent in Portugal than in Spain (χ^2 = 53.153; df = 1; p < 0.001; N = 410), but the opposite happened for waders (χ^2 = 13.050; df = 1; p < 0.001; N = 410) and navigation devices like boats, float tubes or kayaks (χ^2 = 37.588; df = 1; p < 0.001; N = 410) (Figure 3).

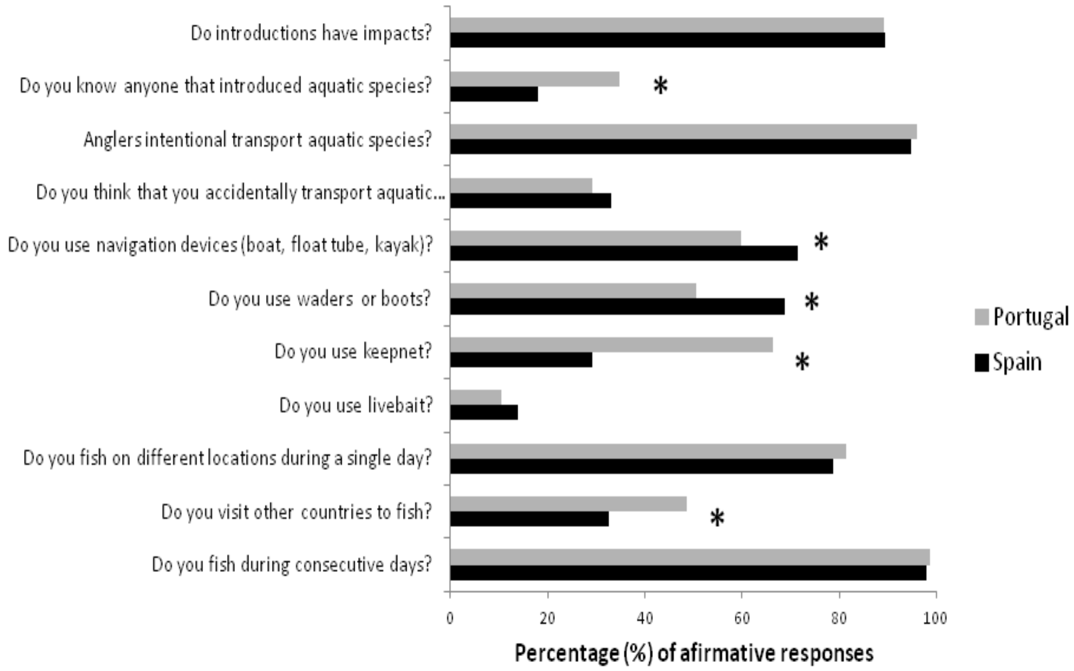

Figure 3 – Percentage of affirmative responses regarding anglers' perceptions and habits. "*" – the proportion of "affirmative" or "negative" responses was different (Chi-square Test: $p < 0.0045$ - below critical value with a Bonferroni correction).

Both for Portugal and for Spain, there were no differences in age distribution between anglers who response affirmative and negative to all topics addressed (Two-sample Kolmogorov-Smirnov test: $p > 0.05$). There are differences between countries on motivation categorical chosen by anglers to justify the introduction of aquatic species into new waterbodies ($\chi^2 = 42.040$; $df = 7$; $p < 0.001$; $N = 410$). For Portugal, the most important motivation classes to justify introductions were Stocking, Lack of knowledge on impacts and Species fishing interest.

Yet, for Spain, Lack of knowledge on impacts appeared in first place followed by Economical motivation, a class with little expression for Portugal. The third place was occupied by Species fishing interest, as occurred for Portugal (Figure 4).

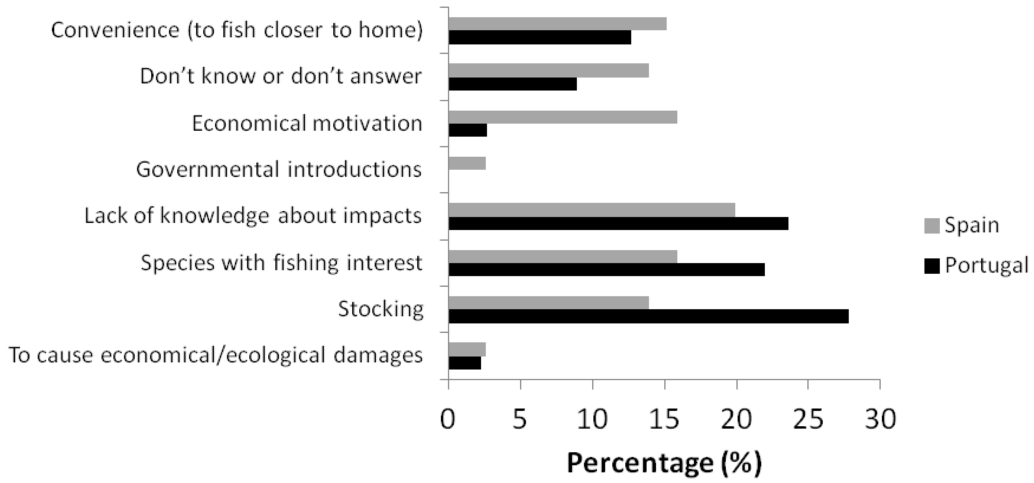

Figure 4 – Percentage of motivational classes indicated by anglers to justify the introduction of aquatic species. There are differences between countries on proportions of motivation categorical chosen by anglers to justify the introduction of aquatic species into new waterbodies (Chi-square Test: $p < 0.001$).

There are differences between Spanish and Portuguese anglers regarding the perception of "environmental impacts of introductions" (Mann-Whitney $U = 17441.0$; $Z = -2.019$; $p = 0.043$; $N = 410$). The majority of the anglers from both countries gave an incomplete response, with only one topic addressed, (47% and 58.3% anglers from Spain and Portugal, respectively). The topic most referred in these responses was the impacts on native species, with anglers being aware that exotic species may predate and compete with native species. The percentage of responses with two topics referred was 20.5%, for both countries and the most frequently missed topic was the effect on ecosystems services. The percentage of wrong or blank responses was 32.5% for Spain and 20.1% for Portugal. Only among Portuguese anglers there were some totally correct responses (1.2%).

For both countries, Portugal and Spain, the non-native largemouth bass (*Micropterus salmoides*) is the species with the largest number of reports of introductions, with a percentage over 20% of all introductions (Table 2). Additionally this species is the most preferred by Spanish anglers (23.5%) and the third by Portuguese anglers (16.5%). The native species of the *Barbus sp.* genus are the most appreciated ones being the most prevalent in Portugal (24.5%) and the third ones in Spain (18.2%). In fact, these native species are the ones with more introductions reported in Portugal (7.7%). Among Spanish anglers there were no reports of introductions of native species. Additionally, in Portugal only 16% of the total reports of introductions were of native species. The non-native and wide-spread carp (*Cyprinus carpio*) is also one the most preferred by anglers, being in the second position in Portugal with 22.7% of the reports, and in fourth position in Spain (16.4%). In Portugal this species, is in the second position of most introduced species. The non-native bleak (*Alburnus alburnus*) has little fishing importance for anglers from both countries, but it occupies the first position in Spain, with the same value as largemouth bass, and the third position in Portugal with 11.3% of the introduction reports. In Spain, a similar situation occurred with the wels catfish *Silurus glanis*. It has little importance for anglers but it is the third most introduced species (Table 2).

Table 2 – Proportion of game fish species preference by Portuguese and Spanish anglers and percentage of respective introductions reported. . N = Native; IS = Invasive Species.

		Portugal		Spain	
Specie	Status	Introduction reported	Preferred game fish	Introduction reported	Preferred game fish
Micropterus salmoides	IS	0.220	0.162	0.250	0.235
Alburnus alburnus	IS	0.113	0.015	0.250	0.009
Barbus sp.	N	0.077	0.245	0.000	0.182
Chondrostoma sp.	N	0.012	0.065	0.000	0.014
Squalius sp.	N	0.024	0.015	0.000	0.000
Cyprinus carpio	IS	0.179	0.227	0.063	0.164
Anguilla anguilla	N	0.006	0.004	0.000	0.000
Rutilus rutilus	IS	0.030	0.008	0.000	0.002
Procambarus clarkii	IS	0.018	0.000	0.031	0.000
Esox lucius	IS	0.030	0.022	0.125	0.200
Sander lucioperca	IS	0.060	0.034	0.063	0.023
Ameiurus melas	IS	0.036	0.000	0.000	0.000
Lepomis gibbosus	IS	0.042	0.003	0.000	0.002
Carassius sp.	IS	0.101	0.044	0.031	0.012
Salmo salar	N	0.000	0.009	0.000	0.002
Silurus glanis	IS	0.012	0.005	0.188	0.023
Liza sp.	N	0.006	0.015	0.000	0.000
Salmo trutta fario	N	0.024	0.111	0.000	0.099
Oncorhynchus mykiss	IS	0.006	0.003	0.000	0.016
Cobitis sp.	N	0.006	0.000	0.000	0.000
Alosa alosa	N	0.000	0.003	0.000	0.000
Alosa fallax	N	0.000	0.001	0.000	0.000
Thymallus sp.	IS	0.000	0.001	0.000	0.000
Salmo trutta trutta	N	0.000	0.008	0.000	0.009
Perca fluvitilis	IS	0.000	0.001	0.000	0.002
Tinca tinca	N	0.000	0.000	0.000	0.005

The use of live bait is restricted to only 14% and 10% of Spanish and Portuguese anglers, respectively (Figure 3). A great proportion of these anglers (Spain - 71%; Portugal - 67%) admitted that they captured live bait organisms in a different place from where they used them. Additionally, more than 70% the anglers (Portugal - 74%; Spain - 76%) which use live bait admitted that they release the bait at the end of the angling session. In both countries, the earthworms followed by the fly maggots are the most used live bait organisms. The invasive live bait most used by Portuguese anglers is the red swamp crayfish (*Procambarus clarkii*) followed by a non-native fish, the bleak (*Alburnus alburnus*). For Spanish anglers these species are also the most important ones but the order is reversed (Table 3).

Table 3 - Live bait use (%) by Portuguese and Spanish anglers. N = Native; IS = Invasive Species.

Bait	Status	Proportion of use	
		Spain	Portugal
Micropterus salmoides	IS	1.33	0.00
Alburnus alburnus	IS	16.00	6.35
Bivalvia (clams)	N	0.00	3.17
Diptera (Fly maggots)	N	20.00	25.40
Chondrostoma sp.	N	2.67	0.79
Athyaephyra desmarestii	N	0.00	4.76
Gastropoda	N	1.33	0.79
Cyprinus carpio	IS	2.67	0.00
Squalius sp.	N	0.00	0.79
Caelifera (Grasshoppers)	N	1.33	1.59
Rutilus rutilus	IS	1.33	0.00
Anura (tadpoles)	N	0.00	0.79
Ensifera	N	1.33	0.79
Procambarus clarkii	IS	9.33	13.49
Ephemeroptera (larvae)	N	0.00	0.79
Diptera (larvae)	N	0.00	2.38
Tricoptera (larvae)	N	2.67	0.79
Gastropoda (land slugs)	N	0.00	0.79
Oligochaeta	N	24.00	27.78
Lepomis gibbosus	IS	6.67	4.76
Tinca tinca	N	4.00	0.00
Salmo trutta	N	4.00	0.00
Oncorhynchus mykiss	IS	1.33	0.00
Cobitis paludica	N	0.00	3.97

The nMDS (Portugal: Normalized Raw Stress= 0.008 – Excellent adjustment (Kruskal, 1964), Dispersion Accounted For (D.A.F.) = 0.991, Tucker's Coefficient of Congruence = 0.996; Spain: Normalized Raw Stress = 0.005 – Excellent adjustment (Kruskal, 1964), Dispersion Accounted For (D.A.F.) = 0.995, Tucker's Coefficient of Congruence = 0. 997) and the hierarchal clustering highlighted for both countries 2 large groups of variables and 4 more restricted groups (Figure 5).

For Portugal, one cluster group (both for d = 15 and d = 4) was formed by anglers who believe that introductions have environmental impacts and that other anglers are responsible for intentional introductions. These anglers also fish consecutive days and several places in the same day. At d = 15, the remaining variables formed another group. This group splits into 3 groups, at a distance equal to 4, being the first formed by anglers that use keepnets and fish in other countries. The second group was formed by anglers with high educational level which use navigation devices like boats and use waders. The third and last group was formed by male anglers which use live bait, know other anglers which introduced aquatic species and that believe that they accidentally transport aquatic species.

For Spain, the two association groups formed at d = 15 were divided into two groups each at d = 4. One of these larger groups incorporates a smaller group characterized by anglers which think that other anglers perform intentional introductions, and that introductions have environmental impacts. These anglers also fish during consecutive days. The second group was formed by anglers which have a high educational level and use navigation devices like boats and waders. These anglers also visit several places when fishing. The other major group was formed by anglers that visit another country to fish and which believe that they accidentally transport aquatic fauna. The last group was formed by male anglers that use live bait and keepnet and that know of other anglers that introduce aquatic species.

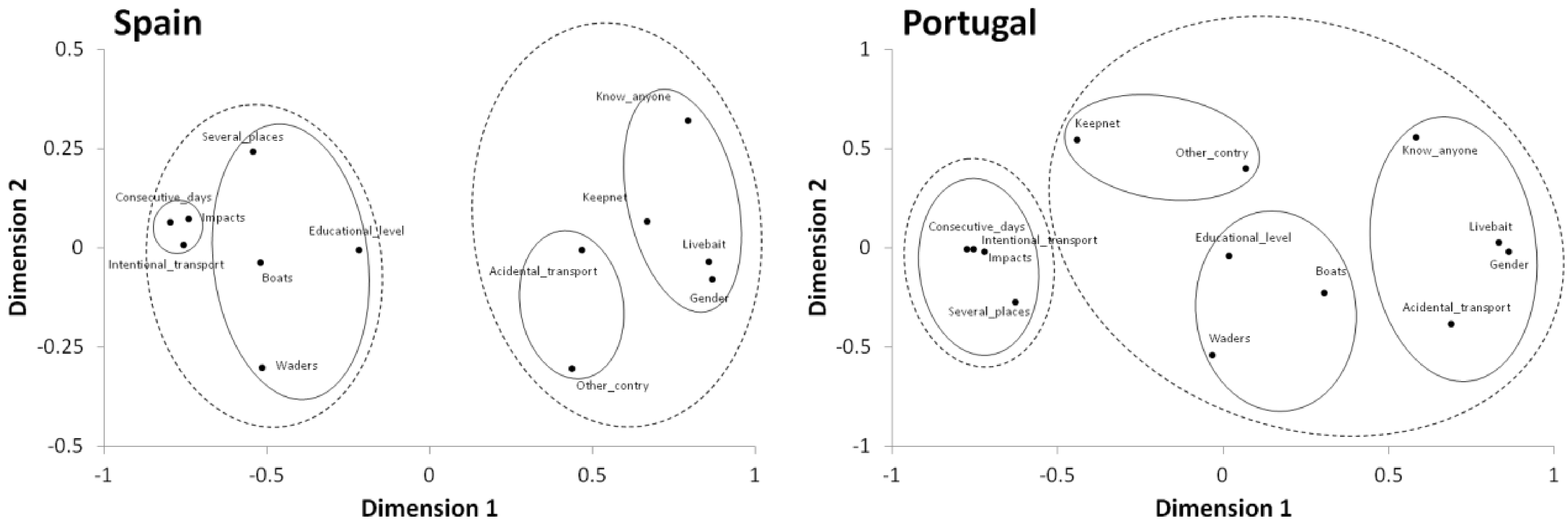

Figure 5 – Association between characteristics, habits, actions and perceptions of Iberian fishermen (Portugal and Spain) that directly or indirectly are related with accidental or intentional transport of invasive fauna. Dissimilarity between variables were defined by nMDS (non-metric Multidimensional Scaling) creating a two-dimension common space. A hierarchical clustering analysis was superposed (Euclidean distances; distance = 15 – dashed line, distance = 4 – continuous line) creating groups of related variables. Thirteen variables were considered, within the following 4 topics – (1) Use of fishing gear: boats (boats, kayaks, float tube); waders (waders, boots); keepnet; live bait. (2) Travelling and angling: Several places; other country; consecutive days. (3) Angler's perceptions of: intentional transport (introductions by other anglers); accidental transport (by themselves); if they "know anyone" (that introduced aquatic species), impacts (of introductions). (4) Angler's characteristics: gender and educational level.

Discussion

This work shows Iberian Peninsula anglers' habits, preferences, motivations and perceptions with direct or indirect implications to aquatic invasions, being the first work of this kind implemented at an international scale. Anglers from Portugal and Spain in general have similar habits and perceptions regarding invasive species. However, Portuguese respondents had a higher level of awareness. In both countries the majority of anglers are informed about the impacts on native species and ecosystems but the socio-economic impacts derived from the ecosystem services loss are missed - only a few Portuguese anglers referred this topic. As expected, anglers with a higher educational level are more aware of introduction impacts and this relation is clearer for Portugal than for Spain. Additionally, this group of anglers is aware that other anglers may promote such actions. So, apparently this group of anglers could be less likely to perform intentional introductions. However, this may not be the case in what concerns unintentional introductions. This group was connected to the use of fishing gear and navigation devices, visited several places and fished consecutive days and these actions may enhance accidental transport.

An internet survey has benefits such as confidently and anonymity, reducing bias on topics related with illegal activities. Regarding the representativeness of our sample, it presented a large age range from teenagers to seniors, a wide range of literacy levels and covered all Iberian Peninsula regions. Although the survey only reached the on-line anglers, both in Portugal and Spain the percentage of population with internet access has been increasing substantially year after year. In 2013, the number of homes with internet connection was 62 and 70%, for Portugal and Spain, respectively (INE, 2014b) and for Spain there was an increase of 5% from 2013 to 2014 (INE, 2014a). Regarding anglers on Portuguese internet forums, there are more than 357000 users, in 11 active forums (Banha et al., 2015) but this number may include some duplication among forums. The last official number of fishing permits disclosed by

Portuguese National Agency (ICNF) indicated 235000 fishing permits in 2000. Therefore, this is an indication that the large majority of anglers are forum users.

An important finding is that, for both countries, anglers tend to prefer invasive species and this may enhance its spread as already proposed by Elvira and Almodóvar (2001). The exceptions are the species of the *Barbus sp.* genus. This could be explained by the large size of the majority of *Barbus sp.* species, in some cases over 1m, but also by their abundance and wide distribution throughout the Iberian Peninsula. In opposition, other native cyprinids presented much smaller lengths and abundances (Doadrio, 2001), and so very little fishing sport importance was attributed to them. The native salmonids, Brown trout and Atlantic salmon, have great sport value throughout the world (Cook and McGaw, 1996; Elliott, 1989) but have a small percentage of preference by Iberian anglers. This may be related to their low abundance and restricted distribution in this region (Cabral et al., 2005; Doadrio, 2001). Thus, anglers tend to prefer large, abundant species, of two major groups: predators, with Largemouth bass and other second order predators like zander, wels catfish, pike; and a second group of large size cyprinids with the common carp and the native species from the genus *Barbus* sp. I

The reported introductions of one fish species seem to be related with angler's preference, indicating a possibility of active intervention in this process. Several authors have mentioned the active role on anglers on deliberate illegal stocking (e.g. (Elvira and Almodóvar, 2001; Lintermans, 2004). Our findings provide objective support to the hypothesis that the probability of introduction depends on the species sports value in the area. In fact, the majority of anglers admit that they know someone having done it or have this perception, being a widespread fact in the angler's community. One exception to this rule is the wels catfish, *Silurus glanis* in Spain that has a great number of reported introductions but is not appreciated by Spanish anglers. This high number of introductions could be related with fishing guide services and tourism. In Spain, namely in the Ebro river, there is an intense touristic activity related to wels catfish fishing, with a great influx of tourists from Central Europe (Binimelis et al., 2007).

This scenario is possibly perceived by Spanish anglers and expressed by a high percentage of responses attributing the motivation of introductions to economic benefits. This is clearly different from Portugal where the economic benefits motivation is much less mentioned. The other exceptions were the invasive *Alburnus alburnus* and the invasive *P. clarkii* which have very little sports value but a large number of reported introductions. In these cases, one possible explanation for spread could be their use as live bait.

The use of live bait has little expression in the Iberian Peninsula and there are restrictive laws in both countries that may explain that fact. Contrary, in Maryland State (USA) more than 60% of the angler community uses live bait (Kilian et al., 2012). Yet, we verified that some Iberian anglers do not respect the law, using illegal live bait. For example in Portugal, the use of the threatened *Cobitis paludica* was banned in 2010, but a great percentage of Portuguese anglers still reported its use. The capture and bucket transport of this bait was previously related with accidental dispersal of invasive species, e.g. of the non-native amphipod *Crangonyx pseudogracilis* (Banha and Anastácio, 2015). Moreover, in both countries, regarding live bait of aquatic origin, anglers tend to prefer invasive species, such as *P. clarkii* and *A. alburnus*. We also noted that the capture of live bait in one location followed by its use in another place is very common.

The use of live bait of terrestrial origin was also reported, namely the use of fly larvae (maggots) and earthworms, and presented a great expression (almost 50% in both countries). The former apparently does not present any environmental risks, but the use of alien earthworms, acquired on angling shops, is a severe problem for terrestrials ecosystems (Keller et al., 2007). In fact, several species of earthworms of African origin are present in Iberian angling shops (Banha, personal observation) and the release or discharge of live bait seems to be a common practice worldwide. Similar results to ours were obtained in the USA, namely in Maryland, where more than 60% of anglers discharge their unused bait when they use crayfishes and fishes (Kilian et al., 2012). A high rate of discharge of earthworms by anglers was also observed in the USA, in the area of Lake Michigan (Keller et al., 2007).

Our survey showed that despite the low number of potentially intentional and accidental (related to live bait use) actions related to the introductions, Iberian anglers have a great mobility which could promote long invasion jumps. This fact, associated to the invasiveness of some species used as bait or preferred as game fish, could explain why some recent invasive species spread so rapidly throughout all the Iberian Peninsula. These great distances involved in illegal transport of fishes were already reported in the Iberian Peninsula (Banha et al., 2015). Moreover, anglers from both countries indicate that the convenience of having the fish species closer to their home locations is one the major reasons for introductions. In both countries, the anglers have the perception that they have an active role on the intentional transport of aquatic species, but they know very little about the causes of introductions, attributing the motivation mostly to lack of knowledge about the impacts of these actions.

Concerning the accidental transport of aquatic species, the majority of anglers believe that this is not relevant or simply that this does not occur. Nevertheless, anglers often fish in several places during successive days, use one or more objects like keepnets, boots, waders and navigation devices (boats, kayaks) and have a peak of fishing activity during late spring/early summer. These facts may potentially contribute to the transport of invasive species in particular, as described for the zebra mussel (*Dreissena polymorpha*) in the Iberian Peninsula (Banha et al. *under review*) but a panoply of other invaders may also be transported such as macrophytes (Johnstone et al., 1985), macrofauna (e.g. amphipods, *Dikerogammarus villosus* (Bacela-Spychalska et al., 2013) microfauna (e.g. *Myxobolus cerebralis* (Gates et al., 2008)) and unicellular organisms (e.g. the freshwater diatom, *Didymosphenia geminatadia* (Kumar et al., 2008)).

Considering anglers' perception of their role as vector for invasive species, the implementation of educational programs about invasive species may be an important step forward in the control of invasions by fishermen. During such programs, it is important to publicize the potential effects of invaders on the loss of ecosystems services and the topic of accidental transport, which have a low rate of awareness. Our findings on angler's behaviors, practices, mobility and activity may be used to model invasion risks and also to improve governmental agencies monitoring and awareness programs. We propose that governmental agencies should not merely impose restrictive rules regarding angling but that these should interact with the angler's community (Johnson et al., 2009). Regular stocking actions with selected native species which present a high anglers' preference, such as the *Barbus* genus, could favor anglers demand for this species instead of invasive fish species.

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Chapter 7 - The role of waterfowl and fishing gear on zebra mussel larvae dispersal

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The role of waterfowl and fishing gear on zebra mussel larvae dispersal

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Abstract

The zebra mussel, *Dreissena polymorpha* (Pallas 1771), is an invasive freshwater species with major negative impacts, promoting changes in ecosystem structure and function and also contributing to economic losses. Navigation has been considered the primary vector of dispersion and little importance has been given to alternative natural (waterbirds) and other human vectors.

Using an experimental approach under field conditions, we evaluated and compared zebra mussel dispersal potential by fishing gear (waders and keep nets) versus mallard ducks (*Anas platyrhynchos*), by examining the adherence and survival rate of zebra mussel larvae on each vector. In addition, we evaluated the survival of zebra mussel larvae under desiccating conditions (i.e., a set of controlled temperatures and relative humidities).

Larvae adhered to all types of vectors and survived desiccation under both laboratory and field conditions and thus appear able to be dispersed long distances overland by both ducks and fishing gear. Specifically, on a per-event basis, fishing gear has a higher potential to spread zebra mussel larvae than ducks. Survival was three times higher on human vectors and the number of larvae attached to human vectors was higher than on ducks. Our findings demonstrate that natural vectors, like ducks, can contribute to the transport of zebra mussel larvae at a local scale. Nevertheless, since vectors related to human activity presented a higher

potential for transport, it is imperative to continue campaigns to raise the awareness of anglers and boaters as well as continue the implementation of legislation to reduce the risk of zebra mussel dispersal.

Keywords: Biological invasions; desiccation; dispersal; *Dreissena polymorpha*; fishing gear; waterbirds.

Introduction

The zebra mussel, *Dreissena polymorpha* (Pallas 1771), is one of the world's worse invasive alien species (Lowe et al. 2000). Due to its filtering capacities, high densities and widespread distribution, *D. polymorpha* alters both the structure and function of the invaded environment, causing large shifts in the flow of energy from planktonic to benthic food webs. It also causes changes in water biochemistry, having been described as an ‘‘ecosystem engineer’’ (Bailey et al. 1999; Jones et al. 1994; Jones et al. 1997; Karatayev et al. 2002; Mayer et al. 2002; Simberloff and Von Holle 1999; Sousa et al. 2009; Strayer et al. 1998), and due to its fouling nature, this species is also responsible for large declines in the populations of native bivalve species (Sousa et al. 2011; Strayer 2008) and for major economic impacts on water-dependent industries and water-supply systems (Connelly et al. 2007; Durán et al. 2012; Pimentel et al. 2005; Sousa et al. 2014).

One of the most recent zebra mussel invasions in Europe occurred in the Ebro River basin, Spain (Altaba et al. 2001), and estimated costs exceeded 13 million euros in almost one decade (Durán et al. 2012). In the Ebro River basin, sport-fishing activities have grown in importance since the late 1970s as anglers from all over Europe come to this area to fish for wels catfish (*Silurus glanis*), carp (*Cyprinus carpio*) and black-bass (*Micropterus salmoides*). One hypothesis for the introduction of zebra mussel in the area is that larvae were brought in water buckets for the transport of live bait (Binimelis et al. 2007), but other vectors associated with fishing, such as boats or anchors, are also possible (Binimelis et al. 2007). Considering that the principal vectors for the large-scale spread of zebra mussels have been shipping and navigation activities (Bidwell 2010; Carlton 1996; Kraft et al. 2002; Minchin and Gollasch 2002), Spanish authorities adopted important measures and new legislation regarding navigation rules, including a restriction on the number of navigable reservoirs and the introduction of disinfection protocols. Actually, these measures together with an intense awareness campaign appear to have helped to slow down the spread of the zebra mussel (but see Johnson et al. 2006). In addition, disinfection has been recommended for all equipment used for freshwater sport and recreational activities including all associated gear that has been in contact with the water, such as life jackets, boots and other fishing equipment (Durán et al. 2010). Additionally, to prevent new biological invasions, the use of live bait (fishes, crustaceans, mollusks and other aquatic organisms) for fishing is now illegal (Ministerio de Medio Ambiente y Medio Rural y Marino 2007). The measures adopted on boat and shipping activities were implemented in agreement with most users, and there has been effective regulation and monitoring by authorities although the monitoring of the disinfection of smaller equipment, like fishing gear, remains difficult (Durán et al. 2010).

A major gap in the knowledge is the spread of zebra mussel by natural overland vectors (Johnson and Carlton 1996). In a scientific, but mainly in a management context, very low importance has been given to waterbirds as a mechanism of post-establishment spread of zebra mussel (Bidwell 2010; Carlton 1993; Johnson and Carlton 1996). Nevertheless, waterbirds have long been considered major vector for the dispersal of aquatic organisms because of their abundant and widespread distribution across the world's wetlands, and their capacity to travel long distances (Figuerola and Green 2002). Moreover, the small size of zebra mussel larvae (200-300 µm) and their high densities would likely favor waterbird transport (Bie et al. 2012; Boag 1986; Figuerola and Green 2002). However, during overland transport, larvae would experience desiccating conditions, and they do not appear to have any adaptations for surviving such conditions, unlike the dispersive stages of some other freshwater organisms (e.g., the ephippia of cladocerans). Thus mortality during transport would be expected to be high.

The aim of our study was to investigate the importance of waterbirds, in relation to recreational fishing gear, on the overland transport of zebra mussel larvae. First, we examined the survival of larvae in the laboratory under desiccating conditions that might cause mortality during transport. Although desiccation tolerance has been examined for juvenile and adult stages (McMahon et al. 1993; Ricciardi et al. 1995; Paukstis et al. 1999), it has never been examined for larvae, despite implications for dispersal. Second, we compared the adhesion of zebra mussel larvae to fishing equipment and bird feathers over different periods of immersion in water and assessed larval survival on these vectors during simulated overland transport. Finally, based on these results, we calculated potential dispersal distances and estimated the relative potential of these vectors as mechanisms of post-establishment zebra mussel spread and find that while all three vectors represent mechanisms of transport, those related to human activities had more potential for spreading zebra mussels, at least on a per-event basis.

Materials and methods

Survival of zebra mussel larvae

A laboratory experiment was performed to determine how long zebra mussel larvae can survive out of water under specific conditions in the absence of wind. We tested two different temperatures 17.5 and 27.5 ºC, which correspond to the lowest and the highest values of the average mean temperatures in summer for the surrounding area of the Ebro River basin. These values were obtained from the governmental agencies “Agencia Estatal de Meteorologia” (AEMET) and “Instituto Meteorologia” (IM) (AEMET and IM 2011). At each temperature we determined survival at three different relative humidities, 30%, 50% and 80% using a refrigerated incubator (IngClimas model EC/E DBO). To obtain a humidity of 80% an ultrasound humidifier [Honeywell BH-860 E] was placed inside the incubator and for a humidity of 30%, 1 kg of silica gel was placed inside the incubator. The relative humidity values used were in the range of values occurring for the same area and season referred above (AEMET and IM 2011). These values are also very similar to the values used by McMahon et al. (1993).

The larvae used in this experiment were collected in the "Galachos de Juslibol" lake (41°42'15,022''N; 0°55'36,717''W; Zaragoza, Ebro River basin) by filtering and concentrating water with a 50-µm-mesh plankton net (KC-Denmark®, length of 125 cm, 30 cm diameter). The samples were collected from a 2.5-m vertical plankton tow taken from a boat. The sample was then concentrated into a 1.5 L plastic bottle, stored at the same temperature as the lake and transported to the laboratory (Facultad de Veterinaria, Zaragoza). This process took approximately 1 hr before the start of the experiment. A single plankton sample was collected on six different days in 10^{th}; 11^{th} of June and 9^{th}, 17^{th}, 16^{th}, 18^{th} of July 2013. In each day, before sample collection, environmental variables in the lake were registered. Water temperature was 26.6 °C (± 0.14 SD), mean pH was 8.1 (± 0 SD), mean conductivity was 794 $\mu s{\cdot}cm^{-1}$ (±9.86 SD) and mean dissolved oxygen was 11.15 g L^{-1} (±1.61 SD). Due to the logistic constraint of having only one incubator, the sample collected on any individual day was used for only a single combination of temperature and relative humidity conditions and thus there was no replication of the six treatments.

The sample was divided in the laboratory into equal parts into a number of cups depending on the abundance of larvae in the sample. The water in each cup was then filtered with a 6-cm-diameter disk of 50-µm Nitex mesh (Sefar Nitex® 03-50/37) so that the larvae were retained on the mesh. The mesh, containing an average of 140 larvae (95% C.I.: 106-173), was then placed into a plastic Petri dish (Fisherbrand, 90x16 mm) and then into the incubator. For each temperature/relative humidity combination, 6 to 12 such groups of larvae were prepared and then sequentially removed after different periods of air exposure ranging from 90 to 360 min. After each dish was removed from the incubator, 15 ml of larvae-free water from the collection site and 0.7 ml of neutral red solution (Rojo Neutro 10G DC Panreac ref. 251619.1605) were added to the Petri dish to ascertain the number of surviving larvae (Horvath and Lamberti 1999). The larvae were left for at least 3 h in this solution after which the mesh was brushed to suspend any remaining larvae. The liquid was then centrifuged for 10 minutes at 1972 *g* (Biofuge

Primo Sorvall) and the concentrated precipitate was then immediately observed under a dissecting microscope (Nikon Eclipse E200; at 100x with cross-polarized light to find larvae [Johnson 1995] and at 400x to see details) or examined later after adding 1 ml of formalin (3%). For distinguishing live and dead larvae, the light was changed to normal light, because the red color of the vital stain can only be seen with non-polarized light. The number of live and dead individuals of the different live stages (veliger and pediveliger) were counted with larvae colored with neutral red considered alive and the larvae not coloured dead (Crippen and Perrier 1974; Horvath and Lamberti 1999). The percentage of live larvae was also quantified in the original water sample at the start of the experiment (i.e., time zero) to determine the initial condition of larvae in the samples of which 62% were veligers and 38% pediveligers.

Adhesion of zebra mussel larvae to waterfowl and human vectors

We investigated whether zebra mussel larvae can adhere to waterfowl (e.g. ducks) and to two different human vectors associated with recreational fishing equipment and compared the frequency of attachment to each vector. This experiment was conducted during 3 days near the town of Mequinenza, on an irrigation pond (41º19'25,801 N; 0º17'31,981 W). The average water temperature was 23.5 ºC (±0.8 SD), average pH was 8.5 (±1.0 SD), average conductivity was 916.2 $\mu s \cdot cm^{-1}$ (±14.3 SD) and dissolved oxygen was 11.4 g L^{-1} (±2.4 SD). We tested two different periods of vector exposure, 1 minute and 10 minutes in the water. These two time periods were selected in order to test the effect of time, with two different orders of magnitude that were short enough to allow replication. Additionally, these values were in the large range of immersion times that angler's keep the gear in contact with water or ducks are on the water (Pers. Obs.). The fishing equipment used was: keep net (bluefish®; nylon, 3 m long; 0.5 cm mesh; 50 cm diameter), neoprene waders (Storm®; size number 45) and a pair of waders boots (Rapala®, size number 45; felt soles). This equipment was chosen because it is commonly used by anglers on the Ebro River. Additionally, this equipment was studied earlier and was considered the type of fishing gear with the highest potential to disperse zebra mussel larvae (Asensio and Carreras 2009). We only used one wader/boot combination and one keepnet because this equipment is industrially made and standardized, presenting little or no variability.

To simulate waterfowl mediated passive dispersal, 3 dead ducks (mallard, *Anas platyrhynchos*; mean weight of 1.15 kg) were used – all ducks were euthanized one day before the experiment, and kept frozen before use. Euthanasia was performed by a registered veterinarian according to the Law of Animal Welfare following the procedure DOMTOR (medetomidine) + Imalgène 1.000 (ketamine) + T-61 (embutramide). In this way, we tried to conciliate the replication and variability of biological elements with ethical issues; we adopted the use of one duck per experiment day causing no observable plumage's damage due to duck manipulation, with no effects on number of larvae adhered throughout the time. This species was selected because of its abundance (Cramp and Simmons, 1977) and high potential for local and regional migrations (Figuerola and Green 2002; Krementz et al. 2011; Rodrigues et al. 2000). To simulate exposure to larvae, the euthanized ducks were pulled with a rope, (a loop on the base of both wings), in the water, individually at a speed of 0.5 m s^{-1}, the highest value of the range typical for duck swimming speed at low metabolic cost (0.35 – 0.5 m s^{-1}; Prange and Schmidt-Nielsen 1970). Each duck was pulled at a depth of 60 cm by a person wearing the waders and boots. During the same period, the keep net was placed into the water, and kept still as anglers normally use it. After the exposure period, all the vectors were removed and individually rinsed in a plastic box for 1 min using a garden hose with larvae-free water (preliminary trials were used to determine the time necessary time to remove larvae). For each exposure period, we ran 30 replicate trials, 10 with each duck, alternating between 1 and 10 minutes trials, over the 3 days (i.e., 60 trials in total). The rinse water from each vector was filtered with a 50-µm-mesh plankton net, and the resulting 100-ml sample was preserved by adding 1 ml of formalin (3%) and kept on ice (during 3 to 21-hr), being immediately processed on arrival to the laboratory. The total number of larvae in the sample was then determined, but due to the differences in the size, shape and material of the vectors, no attempt was made to standardize larval abundance other than on a "per-event" basis (e.g., the total number of larvae adhered to a pair of waders vs. the total number of larvae adhered to a single duck). To determine the natural larval density during the experiment, pond water was collected each day with an 8-L plastic bucket and filtered using the same plankton net used for the rinse water. Each resulting 100-ml sample was preserved by adding 1 ml of formalin (3%) and kept on ice during the transport to the lab. In the laboratory, the number of the different larval stages (veliger and pediveliger) was counted for all samples.

Survival of zebra mussel larvae transported on waterfowl vs. human vectors

Survival of zebra mussel larvae on the three different vectors (duck, keep net and waders) was examined in the field under simulated transport conditions. The larvae were obtained as described for the survival experiment under laboratory conditions. Plankton samples were separated into 27 plastic cups of 100 ml each. Nine cups were used per vector, each one corresponding to a certain transport time on the vector. An additional sample from the water was also used to determine the natural larval density on that day. Before use, all cups were kept at 20ºC without exposure to light. For each trial, water from one cup was slowly poured over each vector (one cup per vector). The vectors were then gently shaken for 4 seconds to remove excess water. Then, the human vectors (waders and keep net) were placed in each respective separate impermeable storing bag (included in each product package) inside the car. The duck (euthanized one day before the experiment, and kept frozen before use) was suspended by taut cords in a position similar to a live duck during gliding flight (extended neck and wings, legs extended near tail) from a metal structure attached to the top of the car. Since the mean flight speed for *Anas* spp. ranges from 60 to 78 km h^{-1} (Welhun 1994), the car was driven at a constant speed of 75 km h^{-1}, during 20, 40, 60, 80, 100, 120, 180 and 240 minutes. The human vectors were tested at the same time (i.e., placed the in the trunk of the car) for all but the 180 min trial, which was replaced by a longer trial of 930 min (but without vehicle movement). The sequence of time trials was random. After the transport period, each vector was individually and thoroughly washed for one minute with a garden hose. The water from each vector was collected individually, filtered with the plankton net and placed into a plastic cup with 1 ml of neutral red solution. All samples were kept refrigerated during transport back to the laboratory where they were examined for live and dead larvae as described above. The proportion of dead larvae was estimated in the original water sample at the start of the transportation trials (i.e., time zero) to determine the initial conditions but also at the middle and at the end of the experiment in the control samples. During the experiments, the average air temperature inside the car was 26.7 ºC (±2.4 SD) and the average air relative humidity was 39.7% (±6.9 SD). Outside the car, the average air temperature was 25.4 ºC (±1.9 SD), the average air relative humidity was 38.1% (±4.8 SD) and the wind speed was 2.7 m s^{-1} (±1.1 SD).

Statistical analysis

Statistical analyses were performed using IBM® SPSS® version 20. Probit analysis was used to calculate the probability of zebra mussel larvae survival and the time for 50% (LT_{50}) and 90% mortality (LT_{90}) in the laboratory experiment. As we did not replicate the different temperature/relative treatments, differences between trials cannot be strictly interpreted being due to the environmental parameters that we manipulated.

The dependent variable from the adhesion experiment, namely the number of larvae adhered to an individual vector, was transformed (log(X+1)) to meet the assumptions of a normal distribution and to achieve homoscedasticity. After this transformation, the influence of vector type and immersion time was analyzed using a two-way ANOVA. Time and vector were considered fixed factors. We use the Tukey HSD post hoc test to determine which pairs of vectors differed significantly and to determine differences between exposures for each vector.

For the experiment on survival during transport, the mean survival time of larvae on the different vectors was calculated using a Kaplan-Meier test. To assess the differences in survival time between vectors, a pairwise-comparison log-rank (Mantel-Cox) test was used. In this last experiment, we used a different statistical analysis from the first experiment for survival analysis due to some censored data (e.g. for human vectors we did not achieve 100% mortality) (Banha and Anastácio 2012).

Results

Survival of zebra mussel larvae

During air exposure, the proportion of dead larvae generally increased logistically with time in all trials (Figure 1). As expected, the larvae survived longer at lower temperatures (17.5ºC) except at the highest relative humidity (RH) value used (80%), with 10% of the larvae alive ("LT_{90}", "lethal time" until 90% mortality) after approximately 3 hr in both trials. The same pattern occurred for the LT_{50} values, except at 80% RH where the time to 50% mortality was twice as long at the higher temperature. This non-intuitive result is likely due to the lack of replication in this experiment (see above). Nevertheless, the results support the idea that increasing mortality occurs at higher temperatures and lower relative humidities. More important, regardless of the treatment conditions, survival of larval stages out of water appears to be only a matter of hours, even under the benign laboratory conditions. The mean percentage of live larvae in the original water sample at the start of the experiment (i.e., time zero) was 92.6% (76-100% range).

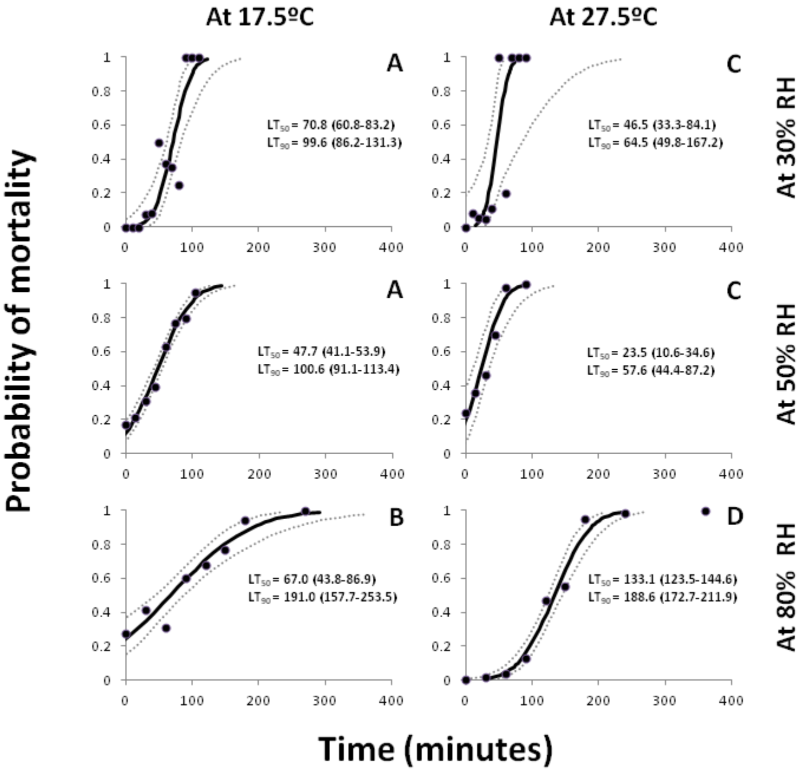

Figure 1 - Zebra mussel larvae mortality as a function of the time spent out of water at six different combinations of temperature (ºC) and relative humidity (%). The black circles are the observed proportions of dead larvae; the black line with the respective 95% confidence intervals (dotted line) was obtained by Probit analysis. Different letters represent statistically significant differences in survival between trials (pairwise comparisons Log Rank (Mantel-Cox): $P<0.005$)

Zebra mussel larvae adhesion to waterfowl vs. human vectors

The type of vector influenced the number of larvae adhered ($F = 9.769$; $df = 2$; $P <0.001$) (Table 1). The Post-hoc Tukey HSD test showed that the mean number of larvae adhered to the ducks was lower than the mean number adhered to both human vectors, with a major difference observed for the keep net (Mean difference = -0.336; standard error = 0.076; $P <0.001$; 95% CI: -0.517 to - 0.156) and followed by waders (Mean difference = -0.188; standard error = 0.076; $P = 0.039$; 95% CI: -0.368 to - 0.008). Indeed, for both submersion periods used, the mean number of larvae adhered to the human vectors was more than the double than for the duck (Figure 2). However, between the two human vectors no significant difference was observed (Mean difference = 0.148; standard error = 0.076; P = 0.130; 95% CI: -0.032 to 0.328). Overall, the immersion time of the vector also affected the number of adhered larvae (ANOVA, F = 4.373; df = 1; P <0.001) (Table 1) with more adhered larvae for the 10-min trials relative to the 1-min trial (Figure 2). However, the post-hoc Tukey HSD test did not show any differences between immersion times for each individual vector (duck: Mean difference = -0.162 larvae adhered/trial; standard error = 0.108; P = 0.661; 95% CI: -0.473 to 0.149; waders: Mean difference = -0.072 larvae adhered/trial; standard error = 0.108; P = 0.985; 95% CI: -0.383 to 0.238; keep net: Mean difference = -0.156 larvae adhered/trial; standard error = 0.108; P = 0.700; 95% CI: -0.466 to 0.155). Moreover, the observed increases in the number of adhered larvae were only 15-40%, in spite of the 10-fold longer exposure. An average density of 12.3 larvae L^{-1} (±9.6 SD; n = 3), with a proportion of 80% of veliger and 20% of pediveligers was found in the pond water.

Table 1 - Influence of vector type and immersion time on log (number of larvae adhered +1) analyzed using a two-way ANOVA.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.246	5	0.849	4.868	0.000
Intercept	154.055	1	154.055	883.089	0.000
Time	0.763	1	.763	4.373	0.038
Vector	3.408	2	1.704	9.769	0.000
Time * Vector	0.075	2	0.037	0.215	0.807
Error	30.354	174	0.174		
Total	188.655	180			
Corrected Total	34.600	179			

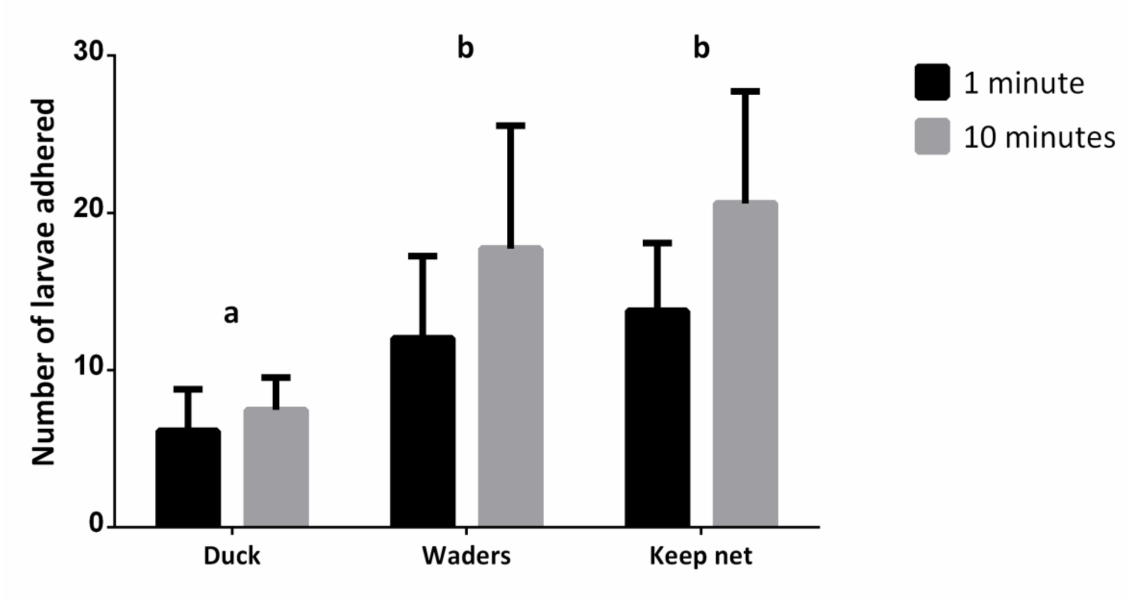

Figure 2 - Mean number of larvae (+SD) adhered to the vectors at two different submersion periods (1 and 10 minutes). Different letters indicate significant differences (P<0.05) between vectors.

Survival of zebra mussel larvae transported on waterfowl vs. human vectors

Survival out of water decreased over time for all vectors (Figure 3). No larvae were alive after 240 minutes on the duck vector, but at the longest time period tested, 930 minutes, 29% of larvae on the waders were alive and 21% were alive on the keep net. The Kaplan-Meier analysis shows a similar mean survival time for the keep net, with 340.7 minutes (Standard Error (SE) = 28.5; 284.9-396.5 minutes 95% CI), and for waders, with 342.2 minutes (SE = 33.7; 276.2-408.2 minutes 95% CI). In fact, there are no differences between the zebra mussel larvae survival time on these two human vectors (Log Rank (Mantel-Cox): X^2 = 3.432; df = 1; P = 0.064). Yet, for the duck, the Kaplan-Meier calculated a mean survival time of 116.0 minutes, almost 3 times lower (SE = 11.0; 94.4-137.5 minutes 95% CI). The pairwise comparisons show that there are differences between the survival time on this vector and on the waders (Log Rank (Mantel-Cox): X^2 = 16.331; df = 1; P = 0.000) but also when compared with the keep net (Log Rank (Mantel-Cox): X^2 = 33.421; df = 1; P = 0.000). The mean number of larvae recovered from each vector was 10 ± 7.2 S.D. (3-24 range) for the duck; 54.1 ± 41.5 S.D. (17-128 range) for the waders and 56.4 ± 38.6 S.D. (17-146 range) for the keepnet. The mean number of larvae in each control cup, was 172.3 ± 95.9 S.D. (70-260 range). The proportion of dead larvae in the controls at the beginning, middle and at the end of the experiment was 16%, 40% and 37%, respectively. 35% of the larvae were pediveligers and 65% were veligers.

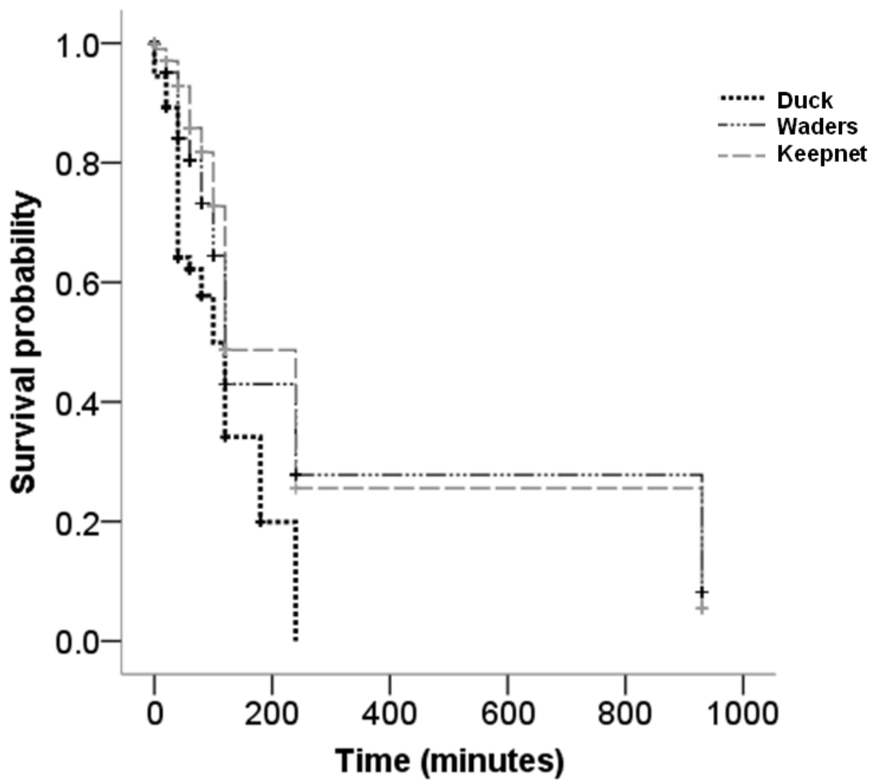

Figure 3 - Survival on the vectors during transport as a function of time.

Discussion

Our work showed that zebra mussel larvae survive out of water for periods that may allow long-distance overland dispersal, i.e. distances over 10 km according to Green and Figuerola (2005). Furthermore, our results are consistent with previous work on this species, namely the negative effect of higher temperatures and low humidity/dry conditions on the survival out of water (Paukstis et al. 1999; Ricciardi et al. 1995). Our findings on larvae survival under desiccating conditions in the laboratory (i.e. LT_{50} = 46.5 at 27.5ºC and 30% RH) are not in accordance with the results obtained for the natural vector in the field (mean survival = 116.0 min. at 26.7ºC and 39.7% RH), when comparable air temperature and relative humidity conditions were used. It is possible that the plumage maintained the moisture, resulting in a higher survival rate on the duck, in spite of the wind which may amplify desiccation.

Waterbirds and fishing gear, like waders and keep nets, constitute potential vectors of transport. According to our findings, and assuming a flight speed of 75 km h^{-1} (Welhun 1994), the zebra mussel larvae could be transported 145 km by ducks, with a 50% chance of survival. Moreover, anglers driving between sites could transport zebra mussel larvae with 50% survival rates for 500-700 km on keep nets and waders, given speed limits on roadways (90 km h^{-1}) and highways (120 km h^{-1}) in Portugal and Spain. As showed by the lab experiment, these transport distances could be affected by weather conditions, increasing in cooler and more humid conditions and decreasing in opposite scenarios. Additionally, these values could also be higher in a real situation since the zebra larvae in our experiments may have suffered some stress or damage due to the capture and transport process. On a per-event basis, our study showed that human vectors (fishing gear) have more potential to spread zebra mussel larvae than natural vectors such as ducks, especially as they transport more larvae (approximately double). Nevertheless, this difference is small when we consider the human vs. natural vector comparison performed by Johnson and Carlton (1996), where there is a three-orders-of-magnitude difference between live well and ducks.

Surprisingly, and contrary to our expectations, the immersion time of the studied vectors doesn't play an important role in the number of larvae that adhered to it. According to our findings, a 1000% increase in exposure time to zebra mussel larvae contaminated water only results in a 15-40% increase in the number of larvae adhered to the vector. Therefore, anything that comes into contact with contaminated water, even for very short periods, needs to be disinfected or thoroughly dried. Furthermore, the number of larvae adhered to a vector seems to be more affected by vector characteristics (e.g. surface) than by the exposure time.

The importance of any particular vector will depend on the stage of the life cycle that is transported, the number of surviving mussels transported per dispersal event, the frequency of such events, and the spatial patterns of vector movement (Johnson and Padilla 1996). Our assertion of higher risks associated with fishing gear relative to waterfowl is largely due to the 3-fold higher survival of larvae when transported by human vectors. The lower survival on waterfowl is likely due to the exposure of larvae to the wind, which will dry plumage faster than on fishing gear kept inside a vehicle. Additionally, the number of larvae that adhered to the human vectors was more than double that of the natural vector, which could also contribute to a higher dispersal risk by these vectors (i.e. higher propagule pressure [Simberloff 2009]). Finally, the maximum speed is higher for human vectors than for birds.

Our findings also show that waterbird-mediated dispersal of zebra mussels may be a more relevant process than previously acknowledged (Bidwell 2010). In fact, our values of larvae adhesion to ducks were 6 to 7 times higher than the ones found by Johnson and Carlton (1996) in which they obtained less than 1 zebra mussel larvae/bird. Considering our findings of adherence and survival rate on ducks, we estimate that each duck may transport 3 to 4 live larvae for more than 100 km.

Compared with our results, recent studies on waterbird dispersal of other aquatic organisms, namely larger crustaceans, have shown a smaller number of individuals adhered per event, 3 to 7 times lower, and shorter transport distances (Águas et al. 2014; Anastácio et al. 2013; Banha and Anastácio 2012; Rachalewski et al. 2013). Short-distance mallard flights are more common than large movements. Mean flight distances are between 1-2 km for foraging away from roost sites (Legagneux et al. 2009), and are 15 km for female mallard movements between diurnal and nocturnal sites (Link et al. 2011). Taking into account our results and the fact that a duck only needs 2 to 12 minutes to fly those distances, we conclude that the zebra mussel larvae transported in such a dispersal event would present a survival probability near 100%. So, transport within these distances is very likely, because vector movements are frequent and the survival rate of the propagules is very high. Therefore, the transport of zebra mussel larvae by ducks (and possibly other bird species) should be considered an important process at a local scale.

The high survival of larvae on the human vectors shows that they can survive overnight. Therefore the implementation of the disinfection protocols by anglers is essential to block the spread of zebra mussels by these vectors. The Ebro River is not only a hotspot for European anglers from countries already invaded by zebra mussel, like France, but also from uninvaded regions of the Iberian Peninsula. Therefore, our findings show that zebra mussel larvae are likely to expand to the northwest or the south-west of the Iberian Peninsula due to transport by anglers. A crucial fact that favors zebra mussel dispersal by the vectors examined here is that the peak of larvae abundance occur in the summer months (Lalaguna and Marco 2008; Mackie 1991), which matches periods of high abundance and activity of both types of vectors, with *Anas platyrhynchos* highest density in August (Holgado and Menárguez 2012) and the summer holidays when many anglers from other counties visit the Ebro River region (Gomez 2005).

Our work provides a first step in investigating the dispersal of zebra mussel by comparing vectors on a “per event” basis. Future studies on the frequency, movement distance and routes of different vectors should be assessed by further fieldwork and would complement our work. This information could be used to model dispersal probabilities and develop spatially-explicit maps of invasion risk. However, our work has some limitations. First, the experimental design of our survival experiments did not include replication of the different treatments (e.g., combinations of temperature/relative humidity in the laboratory; vectors in the field) and thus need to be interpreted cautiously. Also, the use of a dead duck does not replicate exactly the movements or conditions of a live duck. For example, duck feet were not used for swimming and during the simulated flight only gliding was replicated.

As shown in earlier studies on zebra mussels (Johnson and Carlton 1996) and other aquatic invertebrates (Águas et al. 2014; Anastácio et al. 2013; Banha and Anastácio 2012; Frisch et al. (2007); Rachalewski et al. 2013), our findings show that natural vectors, like ducks, can transport of zebra mussel larvae between waterbodies. We suggest that dispersal of larvae by natural vectors may lead to secondary spread and that such natural spread may be less of a "mussel myth" than previously asserted (Johnson and Padilla 1996). However, in order to prevent zebra mussel spread, quantitative and comparative knowledge of the risks of different vectors is needed, both among different vectors associated with human activities (Johnson et al. 2001; Kelly et al. 2013; this study) and relative to natural vectors (Johnson and Carlton 1996; this study). We also conclude that it is essential to continue awareness campaigns for anglers and boaters (Simberloff et al. 2013) as well as the implementation of further legislation to manage human vectors in the context of the risk of biological invasions.

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Chapter 8 - General discussion and perspectives

1. General discussion and perspectives

Human dispersal mechanisms of freshwater fauna are an extensive area of knowledge, with numerous pathways and vectors to be studied all across the globe. Each study about this subject is one more fundamental step to implement biological invasions management or preventive actions. In this thesis, several dispersal mechanisms were studied in a particular geographic area (Iberian Peninsula). However, the implications are global since the focus is on several of the worse invasive species, like *Procambarus clarkii* (chapters 2, 3, 4, 6) and *Dreissena polymorpha* (chapters 6 and 7), that have worldwide distribution. Moreover, some of the studied vectors are present in many other countries.

The articulation of the findings in this thesis gives a global picture of some of the dispersal mechanisms and vectors responsible for the success of the invasive freshwater fauna. The findings regarding *Procambarus clarkii* desiccation survival resistance (chapter 2), were articulated with accidental dispersal by off-road vehicles (chapter 3) and with accidental and intentional transport by anglers (chapters 4 and 6) in order to calculate transport distance involved in each process. In this thesis it became clear that human mediated dispersal of this highly invasive crayfish may occur both intentionally or unintentionally, in many different ways, which may explain the rapid spread across several European countries (Gherardi, 2006). Given that there are large areas worldwide with adequate conditions for this species (Capinha et al., 2011), it is imperative to manage these vectors in order to stop the spread.

The recognition of the importance of angler's web forums as information sources, particularly for the detection of new invasive species (chapter 5), resulted in the implementation of an on-line survey for anglers (chapter 6). The first work using this "web resource" showed the potential of forums and was the trigger to conduct the second study. In sum, both works showed that the access to on-line forums can be an easy and cheap way to collect and deliver information. Additionally, in these two chapters (5 & 6), it was clearly exposed that anglers are intentional vectors for sport fish species, promoting long invasions jumps.

Intentional and accidental human mediated invasive species dispersal mechanisms were studied. In the literature, the major lack of knowledge is reported for accidental introductions (Carlton, 1993). Regarding accidental transport, this work added a new accidental vector for invasive macro fauna - off-road vehicles (chapter 3) - and studied some mechanisms of accidental transport, namely live bait capture (dipnet), crayfish trapping (chapter 4), the use of keepnets and of waders (chapter 7). On the other hand this thesis also added a wider view of angler's role (in the Iberian Peninsula) and also of their perceptions regarding these processes (chapter 6). It is clear that Iberian anglers can promote long-distance accidental transport of freshwater fauna and that their level of awareness for this problem is very low. Additionally (chapter 6), it was demonstrated that larvae of *D. polymorpha*, a highly invasive mollusk, can survive out of water for large periods in summer conditions and can be easily transported for long distances by anglers.

Two different mechanisms for accidental transport of another mollusk, *P. acuta*, were demonstrated. The ability of these freshwater snails to explore different human-mediated dispersal mechanisms could explain their wide distribution range in Europe. The vectors identified for this species, in this thesis, were the use of dipnets for live bait capture (chapter 4) and off-road vehicles (chapter 3). Particularly for the later more studies are recommended since it seems able to promote invasions by other species, namely the golden apple snail (*Pomacea canaliculata* (Lamarck, 1819)) that recently arrived to the Iberian Peninsula (GEIB, 2009). This species is a plague in rice fields, with large negative impacts (Halwart, 1994). Its abundance in the affected areas and the frequent transit of off-road vehicles, makes it urgent to analyze the potential of this vector for the spread of this species.

In this thesis, the intentional dispersal processes were also assessed, namely in chapter 6. A detailed list of introduced freshwater species was obtained and the motivations were assessed.

Additionally, a clear view of the anglers' active role on freshwater species dispersal was obtained, also revealing that invasive fish species are preferred by anglers. This is in accordance with previous, although more theoretic, works (e.g.: Elvira 2001). It was also found that the aquatic species most used as live bait, were also invasive. In fact, the work on this last chapter, was pioneer since anglers' perceptions and habits were studied together and their relations were assessed, contrary to previous studies (e.g. (Goodchild, 2000; Keller et al., 2007; Kilian et al., 2012)) that focused on a particular aspect. Moreover, the methodology in this thesis was also new, due to its applicability to two countries with two different languages. Although these countries share some cultural and social aspects, climatic conditions, native and invasive species and also river basins, different behaviors were found. This may be due to differences regarding biological invasion perceptions or different legislations. Therefore, each preventive action, e.g. legislation, needs to take into account the different realities, even if the species and the vectors to manage are the same. For example, the current efforts to homogenize the European Union legislation about biological invasions, European Union Regulation No 1143/2014 (Official Journal of the European Union, 2014), needs to take inter-country differences into account to achieve its purpose. The same chapter, once again was articulated with the previous one (chapter 5) since the transport distance presumably involved in the illegal introduction of *P. fluviatilis* is in the range of values of anglers movements reported in the survey (chapter 6).

The findings in this thesis can be directly used by managers, in order to prevent the dispersal of a species, by a vector. New legislation could be prepared regulating certain actions such as the need for cleaning potential vectors in transit between waterbodies. Additionally, the results obtained by this thesis could be articulated with geographic information, namely vector abundances and routes (pathways), in order to reveal the areas with a higher risk of invasion. Moreover, these findings could be combined with the environmental suitability of a certain area for a given species. In this way, the areas at higher risk will be the ones that aggregate environmental suitability and an active route or pathways that allow a high transport success of a vector for a particular invader. This could be very useful in the future since until now invasion risk modeling has mostly been focused in aspects like environmental suitability (e.g. (Capinha and Anastácio, 2011; Capinha et al., 2011)) and predictors related to human presence (e.g. (Gallardo, 2014; Gallardo et al., 2015)) or propagule pressure (Leung et al., 2006).

In conclusion, to stop the spread of invasive species it is not enough to act on the major or most recognized vectors since very often the same species can be dispersed by man in many different ways. Therefore, it is necessary to study and compare numerous vectors in order to prioritize management actions. However, it is almost impossible to act upon all vectors, so the goal of management should not be to stop the spread, but to reduce it significantly. This is even clearer taking in account that some invasive species, after being established in an area, can also use natural dispersal vectors like waterfowl. Additionally, governmental, fisheries, water and environmental agencies need to work more closely with water users (e.g. anglers), since their level of awareness and knowledge about invasive species impacts and dispersal processes, should be improved. This seems an essential step to improve the efficiency of restrictive rules and laws.

2. Brief list of management recommendations

From the findings of this thesis I would like to propose the following management recommendations in order to prevent dispersal of freshwater invasive species due to human intervention:

- The vehicles or machinery used in agriculture or recreational activities that contact with water in invaded wetlands need to be cleaned, especially the parts that were in contact with water, mud or macrophytes.
- Anglers should receive information about invasions, namely regarding accidental transport on fishing gear and invasions impacts, but also regarding the legislation, when they obtain their fishing permits. This also applies to professional freshwater fishermen.
- The use of live bait needs to be restricted due to its importance for the spread of invasive species.
- The illegal use of invasive species, like crayfish (*Procambarus clarkii*) or bleak (*Alburnus alburnus*), as live bait needs to be stopped, either by clarifying the legislation or by more efficient communication or surveillance.
- The authorities could promote the aquaculture of a native species that could be sold to anglers (with a permit) restricting the use of live bait to a single species. In this way, the accidental transport or invasive species could be reduced and the discharges of bait could promote the maintenance of the native specie's populations.
- *In situ* cleaning of fishing gear or other materials that contact with water, mud or macrophytes needs to be disseminated as a common practice for anglers or other water users (e.g. divers, boat users, etc...). In some areas invaded by the zebra mussel (e.g. USA or Spain) this practice is compulsory.

3. **Major conclusions**

From this thesis I would like to refer the following major findings:

- The red swamp crayfish (*Procambarus clarkii)* and the signal crayfish (*Pacifastacus leniusculus*) have desiccation survival capacities compatible with long distance dispersal out of water, in adverse summer conditions, presenting an LT_{50} over 10 hours and LT_{90} over 17 hours. Therefore, both accidental and intentional human transport mechanisms have a potential high level of successes.
- Off-road vehicles constituted a viable accidental human dispersal vector for the red swamp crayfish (*Procambarus clarkii*) and the bladder snail (*Physella acuta*). This process presented a low probability of attachment, but a high probability of successful long distance transport, culminated with a likely release of organisms in aquatic environments.
- Live bait capture using dip nets and crayfish trapping constitute viable vectors for invasive freshwater macrofauna dispersal.
- Crayfish trapping promoted the capture of invasive fish species that may be transported intentionally but this technique presented a high efficiency for the target species, *P. clarkii*.
- Dip nets presented a very low efficiency of capture of the target species (*Cobitis paludica*). However small invasive species (e.g. *Gambusia holbrooki* and *Crangonyx pseudogracilis*) were captured very frequently, and may be transported accidentally in bait buckets or by dip nets.
- The importance of the angling web forums as a useful tool to help detection of non-native fish species was demonstrated with the first record of European Perch (*Perca fluviatilis*), a non-native fish in continental Portugal.

- Iberian freshwater anglers have a great mobility, with the majority fishing during consecutive days and visiting several places. However, angler's activity patterns throughout the year were different in Portugal and Spain.
- In Portugal and in Spain the preferred fish species are invasive and its introductions were reported more often than native species. The motivations for introductions were country-dependent.
- A small portion of anglers use live bait, sometimes invasive species, but discharges of unused bait in the water are very frequent.
- The majority of anglers have the perception that introductions have environmental impacts and that anglers have an active role in intentional introductions. However, only a minority is aware of the angler's role on accidental transport of invasive species.
- Zebra mussel larvae desiccation survival is compatible with long distance overland dispersal, since they can survive more than 1 hour out of water in summer conditions.
- Ducks and fishing tackle, such as waders and keep nets, constitute viable vectors for zebra mussel larvae long distance dispersal.
- Comparing human and natural vectors, fishing tackle presented a higher propensity to spread zebra mussel larvae than ducks.

In conclusion, this thesis added important knowledge on Human-mediated dispersal mechanisms of freshwater fauna, with the suggestion of relevant preventive measures.

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Appendix

Appendix 1 (Chapter 6)

The role of angler's perceptions and habits in biological invasions: an international assessment.

Spanish version of the survey

Available on-line on: https://docs.google.com/forms/d/1HCpBzHm2kFQ6rmxbEs-B3QbcmSc8CiAxPKXU5Cvwgm4/viewform

Este cuestionario pretende recoger información sobre los hábitos de pesca en agua dulce. Para ello, son colocadas algunas cuestiones sobre varios aspectos relacionados con la práctica de la pesca deportiva.

Exclusivamente los investigadores involucrados en este estudio Filipe Banha, António M. Diniz y Pedro M. Anastácio, de la Universidad de Évora, tendrán acceso a los datos obtenidos con el cuestionario, siendo las respuestas anónimas y mantenidas en confidencialidad.

Les agradecemos de antemano su participación! Su contribución es esencial para este estudio.

Localidad de residencia:

Municipio:

Provincia:

Comunidad autónoma:

Edad (en años):

Sexo: Masculino Femenino

Nivel de estudios:

Profesión:

Responda a las siguientes cuestiones escribiendo el número de kilómetros que mejor describen su situación.

¿A qué distancia en kilómetros está la zona donde pesca más lejana a su domicilio? __Km

¿A qué distancia en kilómetros está la zona donde pesca más próxima de su domicilio? ___Km

Responda a las siguientes cuestiones señalando con una cruz la opción que mejor describa su situación.

¿Cuál es el mes del año que más va a pescar?

Enero Febrero Marzo Abril Mayo Junio Julio Agosto Septiembre Octubre Noviembre Diciembre

¿Cuál es el mes del año que menos va a pescar?

Enero Febrero Marzo Abril Mayo Junio Julio Agosto Septiembre Octubre Noviembre Diciembre

¿Cuántos días por semana acostumbra a pescar en la época del año en que va más a pescar?

0	1	2	3	4	5	6	7

¿Cuántos días por semana acostumbra a pescar en la época del año en que va menos a pescar?

0	1	2	3	4	5	6	7

¿Con qué frecuencia pesca en río?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Con qué frecuencia pesca en embalse?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Con qué frecuencia pesca dos días seguidos?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Con qué frecuencia se traslada a un país extranjero para pescar?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Cuando pesca, acostumbra a visitar varios lugares en el mismo día?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Cuando pesca, utiliza cebos vivos (peces, cangrejos de río, almejas u otros animales acuáticos)?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

Responda a la siguiente cuestión escribiendo la respuesta en el espacio destinado para ello.

Escriba los nombres de los 3 cebos vivos que más utiliza (ejemplos: alburno, perca sol, colmilleja, cangrejo de río, almeja, etc..). Por favor, hágalo del más al menos utilizado.

____

Responda a las siguientes cuestiones señalando con una cruz la opción que mejor describa su situación.

¿Al final de la pesca libera a los cebos vivos?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Captura a los cebos vivos en un lugar diferente del que pesca?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Cuando pesca usa rejoncillos para retener los peces?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Cuando pesca utiliza botas altas (vadeador) para pescar dentro del agua?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Cuando pesca utiliza medios de navegación (patos, kayaks, barcos)?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Cree que transporta animales acuáticos involuntariamente en su material de pesca (rejoncillos, botas, cubo, barcos, patos, kayaks)?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

¿Cree que otros pescadores liberan intencionadamente peces u otros animales acuáticos en lugares donde no existían?

Nunca	Casi nunca	Muy raramente	Raramente	Pocas veces	Algunas veces	Frecuentemente	Muy frecuentemente	Casi Siempre	Siempre

Responda a las siguientes cuestiones escribiendo la respuesta en el espacio destinado para ello.

Indique el nombre de las 3 especies de peces favoritas que pesca, por orden decreciente de preferencia.____

¿Conoce a alguien que haya liberado algún pez u otro animal acuático en un lugar donde no existía? Si___No ___

Indique la/s especie/s que fueron liberadas. _____________________________

¿Por qué cree que las personas liberaron especies animales acuáticas (peces, cangrejos de río, etc..) en lugares donde no existían?____________________

¿Cree que la liberación de las especies animales acuáticas (peces, cangrejos de río, etc...) en lugares donde no existían tiene consecuencias a nivel ambiental?

Si___ No___

Indique cuáles son las consecuencias. ___________________

Appendix 2 (Chapter 6)

The role of angler's perceptions and habits in biological invasions: an international assessment.

Portuguese version of the survey

Available on-line on:
https://docs.google.com/forms/d/1p6IYnkKpymdTMLOGMhGiK1MNT0ck9UvRYqARK63XerI/viewform

Este questionário pretende recolher informações sobre hábitos de pesca em água doce. Para tal, são colocadas algumas questões sobre vários aspetos relacionados com a prática da pesca desportiva.

Apenas os investigadores envolvidos neste estudo Filipe Banha, António M. Diniz e Pedro M. Anastácio, da Universidade de Évora terão acesso aos dados obtidos com o questionário, sendo as respostas anonimas e mantidas em confidencialidade.

Agradecemos desde já a sua participação! O seu contributo é essencial para este estudo.

Localidade de residência:

Concelho:

Distrito:

Idade (em anos):

Sexo: Masculino Feminino

Escolaridade:

Profissão:

Responda às seguintes questões escrevendo o número de quilómetros que melhor descreve a sua situação.

A que distancia em quilómetros fica o local onde pesca mais longe da sua casa? ___Km

A que distancia em quilómetros fica o local onde pesca mais próximo da sua casa? ___Km

Responda às seguintes questões assinalando com uma cruz a opção que melhor descreva a sua situação.

Qual é o mês do ano em que mais vai à pesca?

Janeiro Fevereiro Março Abril Maio Junho Julho Agosto Setembro Outubro Novembro Dezembro

Qual é o mês do ano em que menos vai à pesca?

Janeiro Fevereiro Março Abril Maio Junho Julho Agosto Setembro Outubro Novembro Dezembro

Quantos dias por semana costuma pescar na época do ano em que vai mais à pesca?

0	1	2	3	4	5	6	7

Quantos dias por semana costuma pescar na época do ano em que vai menos à pesca?

0	1	2	3	4	5	6	7

Com que frequência pesca em rio?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Com que frequência pesca em barragem?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Com que frequência pesca dois dias seguidos?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Com que frequência se desloca a um país estrageiro para pescar?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Quando pesca costuma visitar vários locais no mesmo dia?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Quando pesca utiliza iscos vivos (peixes, lagostins, ameijoas ou outros animais aquáticos)?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Responda à seguinte questão escrevendo a resposta no espaço destinado para o efeito.

Escreva os nomes dos 3 iscos vivos que mais utiliza (exemplos: alburno ou ablette, perca-sol, verdemã, lagostim, ameijoa, etc). Por favor, faça-o do mais para o menos utilizado. ____

Responda às seguintes questões assinalando com uma cruz a opção que melhor descreva a sua situação.

No final da pescaria liberta os iscos vivos?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Captura os iscos vivos num local diferente do que pesca?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Quando pesca usa mangas ou xalavares para reter o peixe?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Quando pesca utiliza botas altas (waders) para pescar dentro de água?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Quando pesca utiliza meios de navegação (patos, kayaks, barcos)?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Acha que transporta animais aquáticos involuntariamente no seu material de pesca (mangas, botas, baldes, barcos, patos, kayaks)?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Acha que outros pescadores libertam intencionalmente peixes e outros animais aquáticos em locais onde não existiam?

Nunca	Quase nunca	Muito raramente	Raramente	Poucas vezes	Algumas vezes	Frequentemente	Muito frequentemente	Quase Sempre	Sempre

Responda às seguintes questões escrevendo a resposta no espaço destinado para o efeito.

Indique o nome das 3 espécies de peixes favoritas que pesca, por ordem decrescente de preferência?____

Conhece alguém que tenha libertado um peixe ou outro animal aquático num local onde esse não existia? Sim____ Não____

Indique a(s) espécie(s) que foram libertadas._______________________________________

Porque é que acha que as pessoas libertam espécies de animais aquáticos (peixes, lagostins, etc…) em locais onde não existiam?____

____

Acha que a libertação de espécies de animais aquáticos (peixes, lagostins, etc…) em locais onde não existiam tem consequências ao nível ambiental? Sim___ Não___

Indique quais as consequências.____

Appendix 3

This appendix is consisted of unpublished results presented at the following international meeting:

Banha F., Anastácio P., Coignet A., Pinet F. and Souty-Grosset C. 2015. Modeling red swamp crayfish dispersal across a network of artificial ponds: the case of the Brenne park (France). European Crayfish conference: Research and Management (9th to 12th April 2015). Landau, Germany.

Modeling red swamp crayfish dispersal across a network of artificial ponds: the case of the Brenne park (France)

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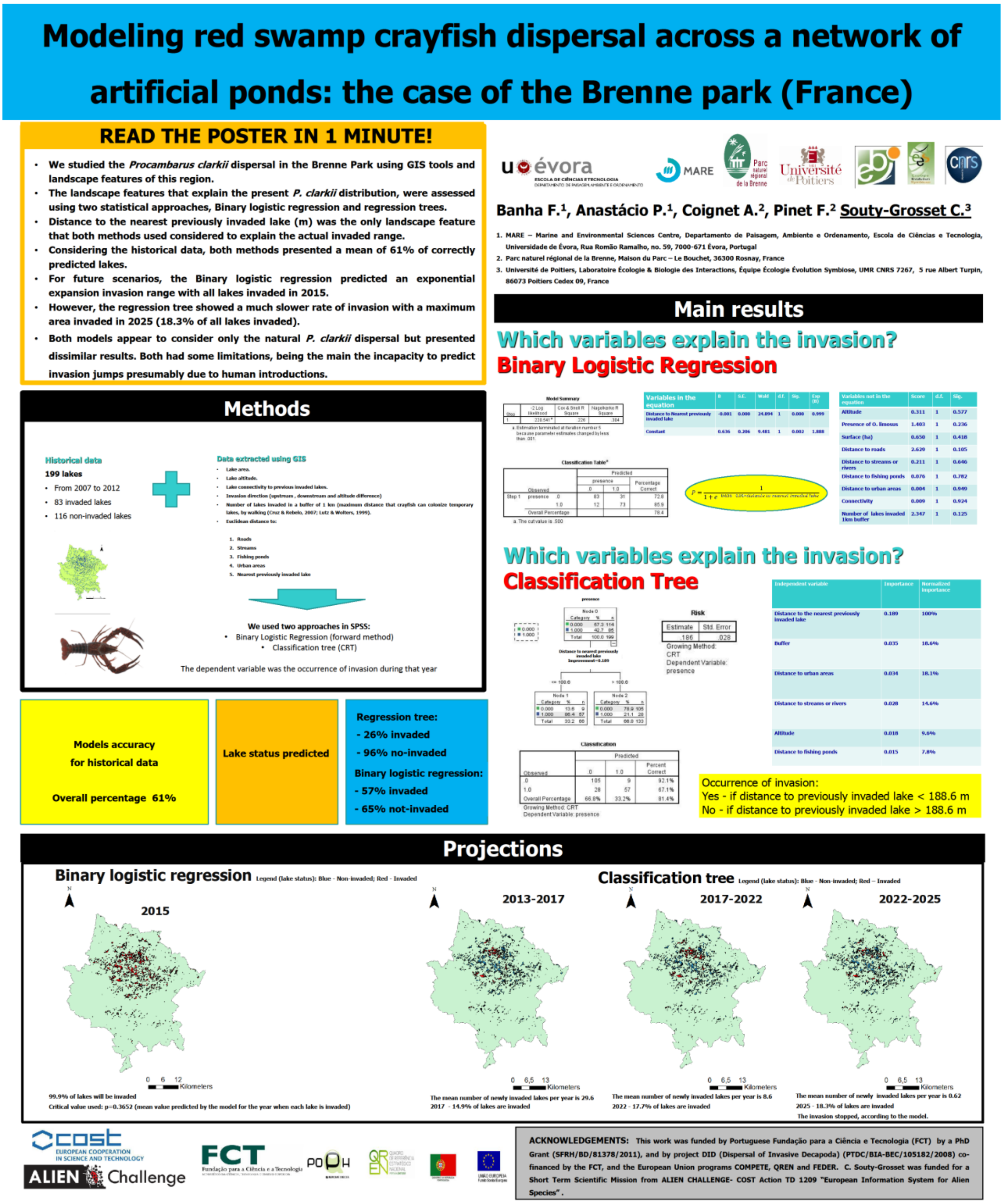
3- Université de Poitiers, Laboratoire Écologie & Biologie des Interactions, Équipe Écologie Évolution Symbiose, UMR CNRS 7267, 5 rue Albert Turpin, TSA 51106, F-86073 Poitiers, France

The "Parc naturel régional de la Brenne" (France) is a wetland area of international importance (Ramsar 1991), named as the land of thousand lakes. In 2007, the Louisiana red swamp crayfish, *Procambarus clarkii*, one of the world's most invasive crayfish, was detected for the first time in this area and rapidly caused severe ecological and economical problems. An exceptional historical invasion record was obtained due to many trapping campaigns executed by both park officers and fish-ponds owners. To help future trapping campaigns and authority's control, we studied the *P.clarkii* dispersal in the Brenne Park using GIS tools and landscape features of this region. The landscape features explaining the present *P. clarkii* distribution, were assessed using two statistical approaches, Binary logistic regression and Regression trees. We found that dispersal (presumably autonomous) between near and connected lakes is the most important process. Human intervention could explain 30.6% of the invaded lakes which are distant, not interconnected, and statistically closer to urban areas. Distance to the nearest previously invaded lake (m) was the only landscape feature that both methods considered to explain the actual invaded range. Considering the historical data, both models obtained presented a mean of 61% of overall corrected predicted lakes. For future scenarios, the Binary logistic regression predicted an exponential expansion invasion range with all lakes invaded in 2015. Yet, the Regression tree showed a slowest rate of invasion estimating a maximum area invaded in 2025 with 18.3% of all lakes invaded.

Key-words : *Procambarus clarkii*, biological invasions, land use, GIS, spread.


Modeling red swamp crayfish dispersal across a network of artificial ponds: the case of the Brenne park (France)
READ THE POSTER IN 1 MINUTE!
We studied the Procambarus clarkii dispersal in the Brenne Park using GIS tools and landscape features of this region.
The landscape features that explain the present P. clarkii distribution, were assessed using two statistical approaches, Binary logistic regression and regression trees.
Distance to the nearest previously invaded lake (m) was the only landscape feature that both methods used considered to explain the actual invaded range.
Considering the historical data, both methods presented a mean of 61% of correctly predicted lakes.
For future scenarios, the Binary logistic regression predicted an exponential expansion invasion range with all lakes invaded in 2015.
However, the regression tree showed a much slower rate of invasion with a maximum area invaded in 2025 (18.3% of all lakes invaded).
Both models appear to consider only the natural P. clarkii dispersal but presented dissimilar results. Both had some limitations, being the main the incapacity to predict invasion jumps presumably due to human introductions.
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Methods
Historical data
199 lakes
From 2007 to 2012
83 invaded lakes
116 non-invaded lakes
Data extracted using GIS
Lake area.
Lake altitude.
Lake connectivity to previous invaded lakes.
Invasion direction (upstream, downstream and altitude difference)
Number of lakes invaded in a buffer of 1 km (maximum distance that crayfish can colonize temporary lakes, by walking (Cruz & Rebelo, 2007; Lutz & Wolters, 1999).
Euclidean distance to:
1. Roads
2. Streams
3. Fishing ponds
4. Urban areas
5. Nearest previously invaded lake
We used two approaches in SPSS:
Binary Logistic Regression (forward method)
Classification tree (CRT)
The dependent variable was the occurrence of invasion during that year
Models accuracy for historical data
Overall percentage 61%
Lake status predicted
Regression tree:
- 26% invaded
- 96% no-invaded
Binary logistic regression:
- 57% invaded
- 65% not-invaded
Main results
Which variables explain the invasion?
Binary Logistic Regression
Which variables explain the invasion?
Classification Tree
Occurrence of invasion:
Yes - if distance to previously invaded lake < 188.6 m
No - if distance to previously invaded lake > 188.6 m
Projections
Binary logistic regression
2015
0 6 12 Kilometers
99.9% of lakes will be invaded
Critical value used: p=0.3652 (mean value predicted by the model for the year when each lake is invaded)
Classification tree
2013-2017
The mean number of newly invaded lakes per year is 29.6
2017 - 14.9% of lakes are invaded
2017-2022
The mean number of newly invaded lakes per year is 8.6
2022 - 17.7% of lakes are invaded
2022-2025
The mean number of newly invaded lakes per year is 0.62
2025 - 18.3% of lakes are invaded
The invasion stopped, according to the model.
0 6,5 13 Kilometers
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