

Conservation status of the freshwater pearl mussel *Margaritifera margaritifera* in Portugal[☆]

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ABSTRACT

Based on new information, the current conservation status of the freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) in Portugal is revised. Between 2010 and 2013 surveys were conducted in eight different rivers to assess distribution, abundance, size-frequency and preferential habitat of this species. Rivers Neiva, Cávado and Terva presented very low abundances (in the Cávado no specimens were found) and showed acute signs of ageing, calling into question the future survival of these populations. Although abundances were low in Rivers Beça, Paiva and Mente, juveniles were present and populations may improve their conservation status in the future, if appropriate management measures are applied. Rivers Rabaçal and Tuela presented the highest abundances of adults and juveniles and these two rivers have the highest potential for the conservation of freshwater pearl mussels in Portugal. However, these two populations have suffered severe declines in several stretches due to the construction of dams in recent years. Given that the Portuguese pearl mussel populations are at the southern range of the species distribution, their conservation should be a priority. This current description of the conservation status of *M. margaritifera* in Portugal can be used as a reference, and guide future research and management initiatives to better conserve this species.

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Introduction

The freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) has a widespread distribution in Europe (from the Iberian Peninsula in the Southwest, to Russia in the Northeast) and was once abundant in many oligotrophic rivers (reviewed in Geist, 2010). However, in the last century many studies have reported extensive spatial declines and reductions in abundance in many rivers (reviewed in Young et al., 2001). Since pearl mussels may be described as indicator, flagship, keystone, and umbrella species requiring almost pristine conditions, and have a life cycle dependent on synchronously declining host fish species (salmon and trout),

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significant conservation interest has been devoted to this species in the last two decades (Geist, 2010). *M. margaritifera* is currently listed as critically endangered in Europe (Cuttelod et al., 2011) and protected in the European Union (EU) under annexes II and V of the European Commission Habitats Directive [92/43/EEC], which require that the EU Member States regularly assess its distribution and abundance and report its conservation status (Reid et al., 2013).

In Portugal, the first comprehensive review of *M. margaritifera* distribution was published by Nobre (1941). In this work the presence of this species is described in several tributaries of the River Douro and also in two other rivers further south: the Rivers Ul and Mira (although the presence in the River Mira is highly improbable since this system is a typical Mediterranean temporary river, which habitat characteristics are, apparently, not suitable for pearl mussels). After this first comprehensive revision, Bauer (1986) visited the Douro tributaries and did not find any individuals; based on this information, Young et al. (2001) stated that pearl mussels were probably extinct in Portugal. A second comprehensive

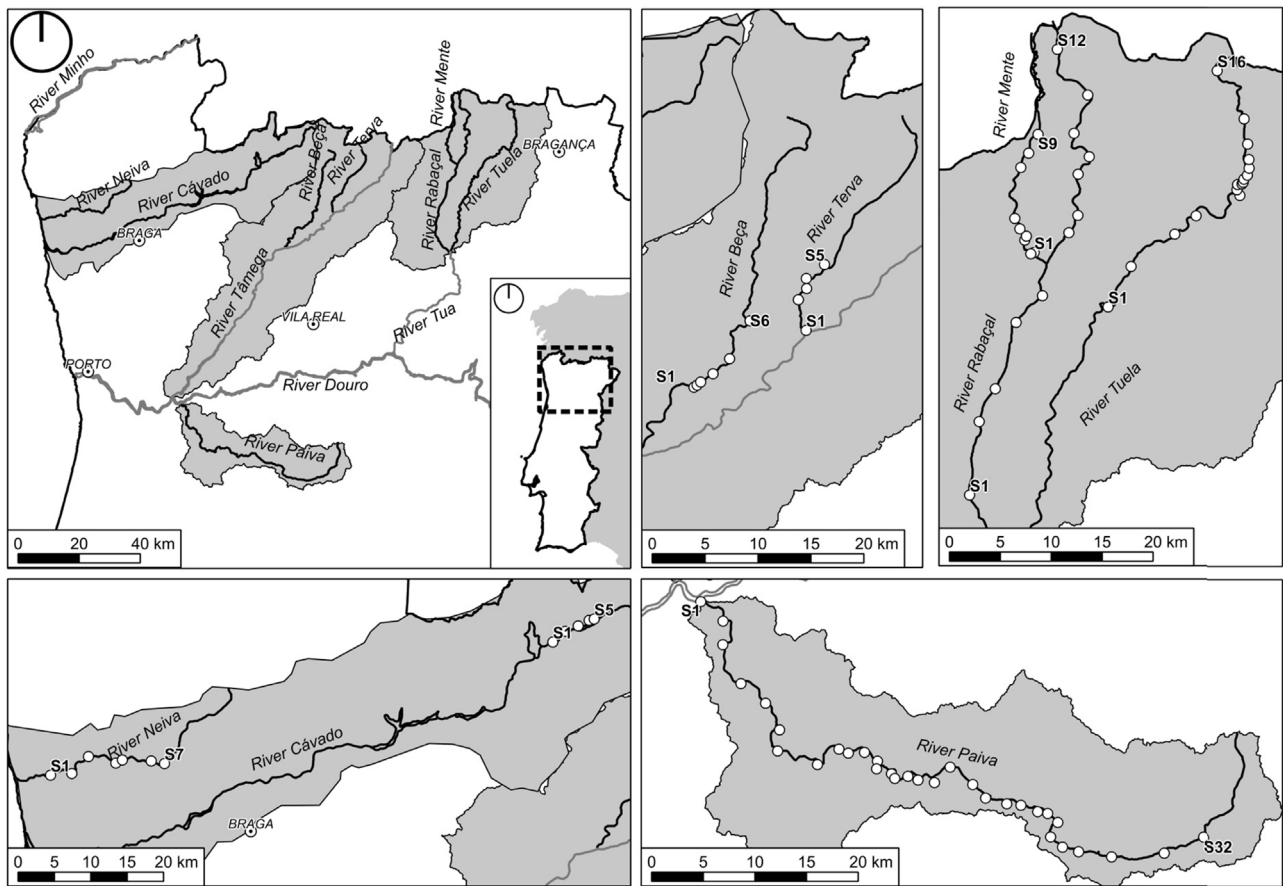


Fig. 1. Map showing the location of sampling sites in the Rivers Neiva, Cávado, Terva, Beça, Paiva, Mente, Rabaçal and Tuella.

review on *M. margaritifera* distribution in Portugal was carried out by Reis (2003) who confirmed the presence of *M. margaritifera* in six different rivers including very small populations in the Rivers Neiva, Cávado and Paiva and large populations in the Rivers Mente, Rabaçal and Tuella. More recently, Varandas et al. (2013) described two more populations in the Rivers Beça and Terva. Currently, all *M. margaritifera* populations are located in the North of Portugal and no reports exist for its presence in the central and southern part of the country.

Given the conservation importance of this species in Europe, including Portugal, the main objective of this study was to summarize information about current distribution, abundance, size-frequency and habitat preference of Portuguese pearl mussel populations. This data, in addition to the discussion of the main threats, can be used as a reference and guide future management initiatives to better conserve *M. margaritifera* in Portugal.

Materials and methods

Study area

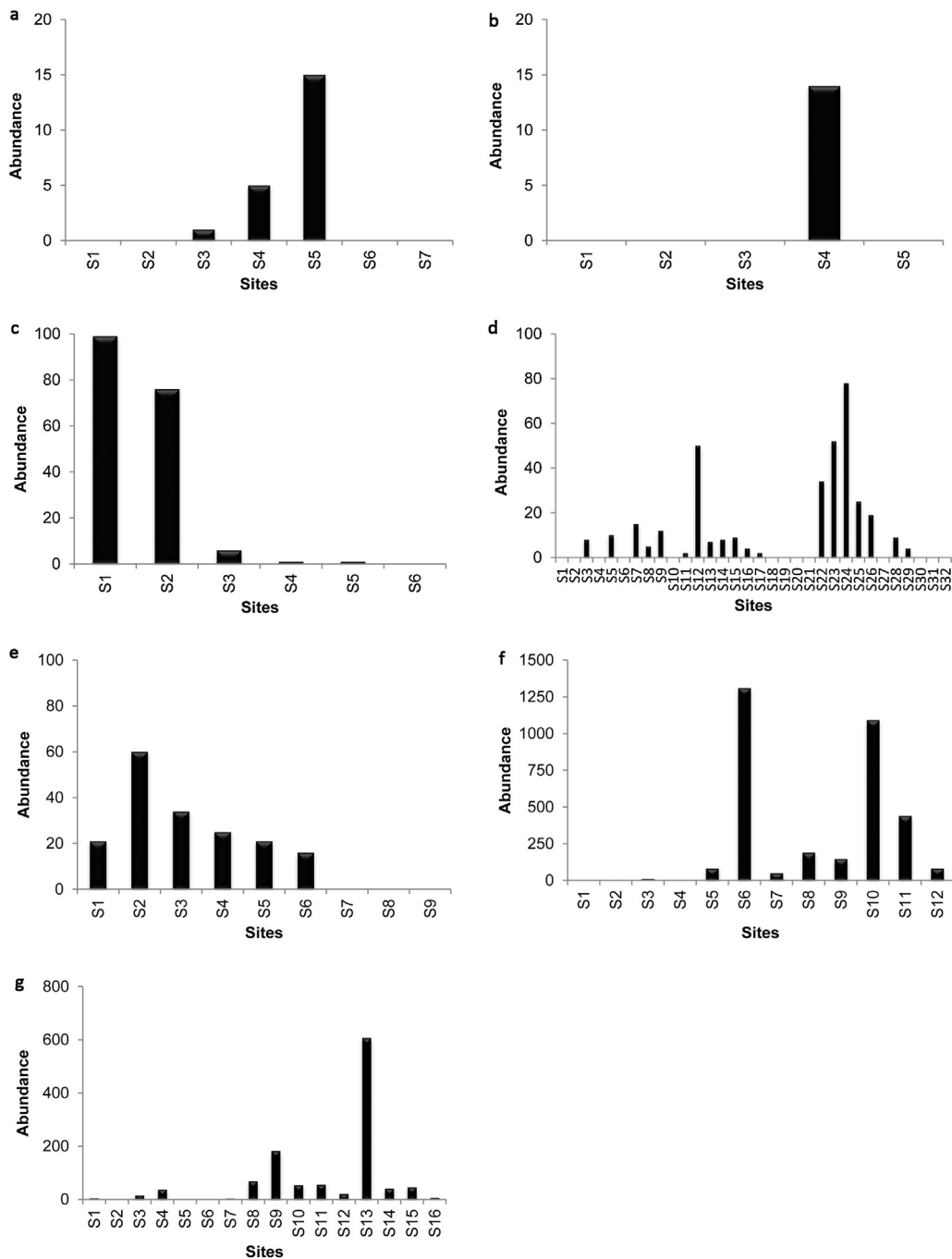
All the surveyed rivers are located in the North of Portugal (Fig. 1). In this region the climate reflects the proximity to the Atlantic Ocean and altitude, ranging from temperate near the coast to Mediterranean more inland (Oliveira et al. 2012). Precipitation levels have high seasonal and inter-annual variability, which is reflected in abrupt changes in the discharge regime of rivers. Floods may occur during winter/early spring, with a gradual decline in river discharge throughout the year, reaching minimal values in the late summer/early autumn. This lower discharge is more pronounced in the continental areas (in comparison to the regions

near the sea) with usually longer droughts. The land use and landscape vary across the studied area, with the largest human settlements and industrial and agricultural activities located in the coastal zones and at lower altitudes. On the other hand, the areas located inland, and at higher altitudes, have low human pressure and are mostly forested (Ferreira et al., 2007).

The study area comprises eight different rivers (Fig. 1). The River Neiva has a length of 45 km, with no dams but with many weirs present. The River Cávado has a length of 118 km and several large dams for hydroelectric production are present within the basin. The Rivers Beça and Terva are tributaries of the River Tâmega; Beça has a length of 46 km and Terva of 27 km (for a detailed characterization, see Varandas et al., 2013). The River Paiva has a length of 108 km (for a detailed characterization, see Sousa et al. 2013). The Rivers Mente, Rabaçal and Tuella are tributaries of the River Tua and have a very low human pressure. The River Mente has a length of 57 km, River Rabaçal 88 km and River Tuella 102 km; four dams are present in the Rivers Rabaçal and Tuella, two in each river.

Sampling strategy and data analysis

Assessment of the *M. margaritifera* conservation status was carried out between 2010 and 2013, during spring and summer months. Surveys included a total of 92 sites (7 sites in River Neiva, 5 in River Cávado, 5 in River Terva, 6 in River Beça, 32 in River Paiva, 9 in River Mente, 12 in River Rabaçal and 16 in River Tuella; Fig. 1). For each site, a river stretch with a total length of 100 m was visually surveyed using glass bottomed viewers and snorkelling. However, in rivers with very low abundance, more extensive lengths were surveyed; in River Neiva S1, S2, S3, S6 and S7 were surveyed for 250 m and S4 and S5 for 500 and 3000 m, respectively; in River



habitat where the specimens were found. The first three attributes were recorded using qualitative scales: four categories for cover (0=absent, 1=roots or vegetation, 2=cobbles or boulders and 3=bedrock); three categories for substrate (1=roots, 2=sand and gravel and 3=cobbles, boulders or bedrock) and six categories for current velocity (qualitative data varying from 0 to 5 where 0 represents null and 5 very high velocity). The water depth and the nearest distance to the river bank were measured with a ranging pole and a tape-measure, respectively.

Results

M. margaritifera was found in seven Portuguese rivers. Of the 92 sites surveyed, 59 (64%) contained mussels (none in River Cávado, 3 in River Neiva, 1 in River Terva, 5 in River Beça, 19 in River Paiva, 6 in River Mente, 10 in River Rabaçal and 15 in River Tuela).

Rivers Neiva (Fig. 2a) and Terva (Fig. 2b) presented low abundances, and individuals were scattered and restricted to very small patches, particularly in the River Terva where specimens were only found at one site. A total of 21 mussels were found in River Neiva and site S5 presented the highest number with 15 individuals per 3000 m of river stretch. A total of 14 individuals per 900 m of river stretch were found in River Terva in S4. Rivers Beça (Fig. 2c), Paiva (Fig. 2d) and Mente (Fig. 2e) possessed intermediate mussel abundances. However, in the Rivers Beça and Mente the individuals were restricted to downstream areas. In River Paiva, the species was present almost throughout the entire river length, with the exception of a few kilometres near the mouth and the head. A total of 183 individuals were found in River Beça where S1 presented the highest abundance with 99 individuals per 750 m of river stretch. A total of 353 individuals were found in River Paiva, being S24 the site which contained the highest abundance with 78 individuals per 100 m of river stretch. A total of 177 individuals were found in River Mente, with S2 containing the highest abundance with 60 individuals per 100 m of river stretch. The highest abundances of pearl mussels in Portugal were found in Rivers Rabaçal (Fig. 2f) and Tuela (Fig. 2g), with the species being present in all surveyed sites except in the areas subjected to the influence of dams (S4 in River Rabaçal and S6 in River Tuela) and in S1 in River Rabaçal. A total of 3411 individuals were found in River Rabaçal, with the highest abundance of 1310 individuals per 100 m of river stretch in S6. A total of 1149 individuals were found in River Tuela, with the

highest abundance with 607 individuals per 100 m of river stretch in S13.

The population size-frequency in Rivers Neiva, Terva and Beça showed a bias towards large (and old) specimens, including a higher representation of individuals larger than 90 mm, mainly in Rivers Terva and Beça (Fig. 3). Juveniles were found in Rivers Neiva and Beça but not in River Terva. In River Paiva the 80–90 mm size class contained most individuals, with very few juveniles found (Fig. 3). In Rivers Mente, Rabaçal and Tuela the great percentage of individuals were in the 60–70 mm size class (River Mente) or 70–80 mm (Rivers Rabaçal and Tuela), with larger numbers of juveniles present, when compared to other rivers (Fig. 3). The smallest individual collected measured 18.0 mm (River Rabaçal) and the largest 123.0 mm (River Beça). There was a clear difference in the mean length of individuals at the different rivers ($H=651.6$; $p<0.001$), with the individuals from Rivers Mente, Rabaçal and Tuela being clearly smaller than individuals from other rivers, especially from Rivers Terva and Beça (Fig. 3).

The majority of *M. margaritifera* specimens were found in sand and gravel substrate in areas typically covered by riparian vegetation and current velocities between categories 1 and 3 during spring/summer conditions (Fig. 4). Regarding water depth, mussels were found at depths less than 100 cm with more than 80% of the bivalves being collected within 3 m from the banks, and very few in the middle of the river channel (Fig. 4).

Discussion

Conservation status

Until the beginning of the 20th century, *M. margaritifera* was a common species in the North of Portugal, colonizing several tributaries of the Douro and Vouga basins (Nobre, 1941) and possibly also other rivers draining directly to the Atlantic Ocean such as the Minho and Lima (populations still exist in these two rivers and upstream tributaries on the Spanish side (Lois et al., 2014) and some unpublished surveys also report their historical presence within the Portuguese borders). Due to anthropogenic disturbance, mainly in the form of habitat loss, habitat fragmentation and pollution, *M. margaritifera* has declined rapidly or disappeared from several Portuguese rivers. Indeed, Bauer (1986) was unable to find any specimen in Rivers Ferreira (12 sites surveyed), Sousa (8 sites

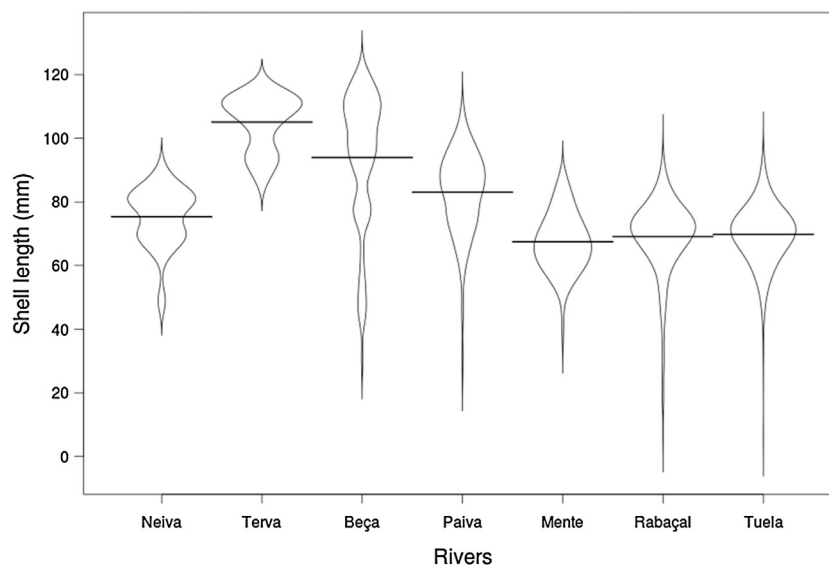


Fig. 3. Shell length distribution of *M. margaritifera* in Rivers Neiva ($N=21$), Terva ($N=14$), Beça ($N=183$), Paiva ($N=353$), Mente ($N=177$), Rabaçal ($N=3411$) and Tuela ($N=1149$). Beanplots show mean values (horizontal line) and the wider the shape at a given shell length, the more specimens are associated with that value.

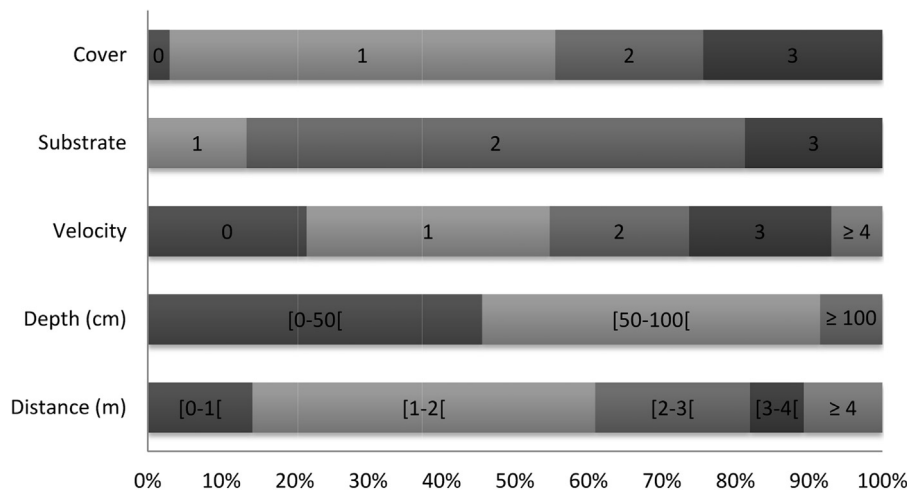


Fig. 4. Percentage of *M. margaritifera* individuals in relation to habitat characteristics ($N=1947$): cover (qualitative data, 1 = roots or vegetation, 2 = cobbles or boulders and 3 = bedrock), dominant substrate (qualitative data, 1 = roots, 2 = sand and gravel and 3 = cobbles, boulders or bedrock), current velocity (qualitative data, varying from 0 to 5 where 0 represent a null velocity and 5 a very high velocity); depth (cm); and nearest distance to the bank (m).

surveyed), Paiva (10 sites surveyed) and Tâmega (3 sites surveyed), which lead Young et al. (2001) to conclude that the species was possibly extinct in Portugal. The main causes for this supposed extirpation were unknown, although eutrophication was the main candidate for Rivers Ferreira, Sousa and Tâmega but not for Paiva (Bauer, 1986). However, during 2001 and 2002, Reis (2003) carried out a nationwide survey (including 240 sites in 131 rivers and streams) to assess the distribution of this and other freshwater bivalve species. This author was able to confirm the presence of *M. margaritifera* in six Portuguese rivers and also studied its population density and structure for some of these rivers (see further discussion below). The present study provides a more recent appraisal of the conservation status of pearl mussels in Portugal, re-surveying previously identified populations and reporting the main findings concerning recently described populations (Rivers Beça and Terva; Varandas et al., 2013).

The declining rates described for Portuguese populations follow the general trends of many European *M. margaritifera* populations (reviewed in Geist, 2010). In comparison with the surveys made by Reis (2003), this study shows a higher abundance and more extensive spatial distribution in the Rivers Neiva and Paiva. These differences could be related to a higher sampling effort in the present study, which surveyed almost the entire length of these two rivers, in comparison with the restricted surveys performed by Reis (2003). Such an explanation seems more realistic than assuming the recovery of these populations in such a short time period. Additionally, and contrary to Reis (2003), no specimens were found in the River Cávado despite an intensive survey conducted on five different sites where specimens were previously located. Populations in Rivers Beça and Terva were recently discovered (Varandas et al., 2013) and for that reason, it is impossible to make any kind of comparison with earlier studies. Finally, the populations of Rivers Mente, Rabaçal and Tuella seem to have a lower abundance than those reported by Reis (2003). This situation may be explained by two factors: (1) the different methodology used in the two studies; and (2) an extensive decline due to increase of human disturbance in these rivers, mainly due to construction of dams (see below for further discussion). Despite the possible increase of human disturbance in recent years, the largest populations still occur in the Rivers Rabaçal and Tuella, both of which flow through areas with low human pressure.

The pearl mussel population size-frequency in the Rivers Neiva, Terva, Beça and Paiva revealed acute ageing, with a prevalence of individuals measuring over 80 mm in length. This situation has

already been described for many Iberian and other European populations and is the major factor concerning the survival of this species (Álvarez-Claudio et al., 2000; Hastie et al., 2000; Geist, 2010; Österling et al., 2010; Hastie, 2011; Lois et al., 2014). Nevertheless, with the exception of the River Terva, a few juveniles were present. On the other hand, the Rivers Mente, Rabaçal and Tuella, although dominated by larger animals, presented a high number of juveniles, which indicate recent recruitment and show that these populations are still functional. Although the current study sampled a much larger number of specimens, a comparison with Reis (2003) show that the population structure is still very similar in Rivers Paiva and Tuella and slightly different in the Rivers Mente and Rabaçal. In the River Mente, the specimens are currently smaller, dominated by the 60–70 mm size class instead of 70–80 mm, whilst in the River Rabaçal, individuals are larger, dominated by the 70–80 mm size class, instead of 60–70 mm, with much lower prevalence of sizes classes smaller than 60 mm.

In the surveyed rivers, *M. margaritifera* were predominantly found in clean oligotrophic river stretches, colonizing areas near the banks at low depths, in sand and gravel sediments, with a high degree of riparian vegetation cover and a moderate-fast flowing habitats of rapids, runs and riffles. Similar results have also been widely described for other European populations (Hastie et al., 2003a; Morales et al., 2004; Outeiro et al., 2008; Geist, 2010).

Main threats

Contrary to some European countries (e.g. Finland, Ireland, Northern Ireland, Scotland and Russia; Beasley and Roberts, 1996; Cosgrove et al., 2000; Makhrov et al., 2014) pearl fishing was never a problem for Portuguese populations and there is no report of this activity in the studied rivers. However, some anecdotal information provided by local older people report the casual use of these mussels as a source of protein for human consumption. Even so, it seems that overexploitation may have a minor influence explaining the declining trends in Portuguese populations.

Introduction of non-native species seems also to be a minor problem for the survival of *M. margaritifera*. Although the Asian clam *Corbicula fluminea* is present in some of the surveyed areas (first 50 km of the River Paiva and downstream areas of Rivers Rabaçal and Tuella) its density was very low, and it is possible that this species only has minor impacts on *M. margaritifera* (Sousa et al., 2013). The North-American signal crayfish *Pacifastacus leniusculus* (Dana, 1852) recently invaded the upper parts of Rivers Rabaçal

and Tuela, which may represent an additional threat to normal *M. margaritifera* recruitment due to juvenile mussel predation. Some non-native fishes are also present in the surveyed rivers (e.g. *Gobio lozanoi* (Daodrio and Madeira, 2004) and *Lepomis gibbosus* (Linnaeus, 1758)), which may affect *Salmo trutta* (Linnaeus, 1758) populations and interfere in the relationship of pearl mussels and their hosts. Anyway, the impacts of these invasive species on *M. margaritifera* remain speculative and future works should be conducted to assess possible effects.

Since Portuguese pearl mussel populations are at the edge of the species distribution, it is expected that predicted impacts resulting from climate change might affect these populations (Hastie et al., 2003b). In theory, two outcomes mediated by climate change may be more problematic: (1) if temperature increases, it is expected that populations located at the southern edge of their distribution, may be the first to disappear because these populations are probably at the limit of their physiological capacity (Sousa et al., 2013) and (2) if extreme climatic events increase in frequency and severity, this may impose a great problem to pearl mussel populations. Indeed, floods and droughts may increase in the future and this situation may be problematic for pearl mussels. Mortalities of *M. margaritifera* in several Portuguese rivers were described after floods (Sousa et al., 2012) and the same is possible to occur during droughts (Sousa et al., 2008).

Water deterioration, due to pollution and changes in land use, has been pointed out as a major threat to *M. margaritifera*, resulting in drastic declines (Young et al., 2001; Geist, 2010). In Portugal, rivers such as Ferreira and Sousa, known to have been colonized by very dense populations of pearl mussels at the beginning of the 20th century (Nobre, 1941) are nowadays heavily polluted. As a consequence, *M. margaritifera* completely disappeared from these rivers since at least the mid-1980s (Bauer, 1986). Currently, the Portuguese populations are restricted to rivers with very low human pressure and with low levels of pollution. The exception is River Terva, since this river is known to have some eutrophication problems (Varandas et al., 2013). In addition, some stretches in the Rivers Neiva and Paiva still receive sewage discharges.

Habitat loss and fragmentation is usually considered the most important threat to freshwater mussels, including *M. margaritifera* (Cosgrove and Hastie, 2001). Direct physical habitat disturbance resulting from channelization of rivers or the presence of impoundments, usually result in pearl mussel declines and a decrease in connectivity, including in the host fishes (Mueller et al., 2011; Addy et al., 2012). Particularly important in Portugal are the changes mediated by the presence of recently constructed dams and weirs in several of the rivers surveyed, which may be responsible for severe declines in pearl mussels. For example, the two dams present in the River Rabaçal were recently constructed (were not present during the sampling made by Reis (2003)) and are 1.5 km upstream of S4 and 2 km downstream of S5 (this last dam also affects River Mente). On the other hand, S6 is located upstream of the influence of this second dam in the River Rabaçal. A comparison of the pearl mussels' abundance at S4 and S5 with S6 and further upstream sites shows a clear difference. The main driving mechanisms responsible for the great decline in *M. margaritifera* near these physical structures are not known but may include alterations in depth, flow, temperature, sediments, organic matter, among other factors (Vaughn and Taylor, 1999; Addy et al., 2012). For a species such as *M. margaritifera*, which usually colonizes sites with oligotrophic and lotic conditions, their presence in the areas upstream of these physical structures is highly improbable due to increased water depth, change from lotic to lentic conditions, accumulation of fine sediments and changes in oxygen and temperature profiles. Many alterations may also occur downstream of these obstacles and may include direct effects through physical stress and indirect effects through changes in habitat, food and fish-host availability (Vaughn

and Taylor, 1999; Addy et al., 2012). Releases from impoundments often result in both abnormally high and low flows (Vaughn and Taylor, 1999). High water velocities can displace adult and juveniles and, possibly more important, impair recruitment. In contrast, extended periods of low flow below impoundments can result in mussel mortality due to stranding because *M. margaritifera* has very low mobility and a clear preference for the colonization of sites near the banks. These changes in flow regimes can also alter the sediment stability and dynamics affecting both adults and juveniles (Geist and Auerswald, 2007). Some studies also showed that temperature may change in up and downstream areas of these impoundments (Vaughn and Taylor, 1999), and this situation may also affect the reproduction of pearl mussels. Finally, the presence of dams may affect the mobility of fish hosts, which consequently may impair the reproduction of pearl mussels.

Conservation measures

M. margaritifera has a life span in excess of 100 years, although in the Iberian Peninsula the species "only" reaches around 60 years (Outeiro et al., 2008); sexual maturity is attained at a relatively late stage and the dispersal capacity is reduced. While adult mussels are sedentary and have a limited tolerance for any kind of disturbance, the juveniles are highly dependent on the substrate quality and stability and have very poor survival rates (Geist, 2010). The glochidia larvae are obligate parasites of salmonids, which have experienced a synchronous decline over the last decades (Geist, 2010). All these features make this species highly vulnerable to habitat disturbance, a fact that makes conservation management much more complex (Beasley et al., 1998). On the contrary, and due to their extended life span, if adults can survive in present disturbed conditions, conservation may be possible if the appropriate management measures, that guarantee effective reproduction and future recruitment, are implemented (Hastie, 2011). In the same vein, and due to the high fertility, captive breeding and future stocking in the wild can be a viable solution if the suitable conditions are restored (Preston et al., 2007; Bolland et al., 2010; Thomas et al., 2010; Gum et al., 2011).

Given this background, two main options for the conservation of *M. margaritifera* in Portugal are possible, which encompass *in-situ* and *ex-situ* conservation measures. For the more abundant populations of Rivers Paiva, Mente, Rabaçal and Tuela the main option should be *in-situ* conservation, protecting these populations against the main threats described above. Some of the sites colonized by this species are already inside protected areas. The Natural Park of Montesinho covers the upstream areas of Rivers Mente, Rabaçal and Tuela where the highest total abundance and presence of juveniles were reported. As such, it is crucial that these ecosystems should be considered for full protection of the Portuguese breeding stock. Similarly, the River Paiva is classified as a Site of Community Importance within the Natura 2000 network. However, as far as we know, there are no special actions focused on the protection of freshwater pearl mussels or even a special attention devoted to conservation of these aquatic ecosystems, with the exception of the Ecotone Life+ project partly devoted to the conservation of pearl mussels in the River Paiva. In the Rivers Cávado (where no specimens were found recently), Neiva, Terva and Beça, populations are restricted to small areas and with very low abundances; *ex situ* conservation measures should complement *in situ* interventions. In this regard, captive breeding should be an option in order to release juveniles in appropriate habitats or in restored stretches of these rivers. Some successful captive breeding programs for *M. margaritifera* already exist in Europe, and so, these techniques are already available to be used in Portugal, taking in account the best practices that should include genetic information, careful determination of habitat requirements (from the macro to the microscale) and assessment of suitable food resources (e.g. Preston et al., 2007;

Bolland et al., 2010; Gum et al., 2011; Wilson et al., 2012; Eybe et al., 2013; Scheder et al., 2014).

In conclusion, full legal protection, mitigation of the main threats, heightened awareness, applied *in-situ* and *ex-situ* conservation measures and information on the distribution, basic ecological traits (e.g. reproduction rates, fecundity, age at maturity, host infestation rates, growth rates and life span) and genetics of *M. margaritifera* are the main options to conserve Portuguese populations. The data collected during this study provides an up to date assessment of the conservation status of *M. margaritifera* in Portugal and can be used as a reference, and guide future research and management initiatives to better conserve this species.

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