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Ecological Status of a *Margaritifera margaritifera* (Linnaeus, 1758) Population at the Southern Edge of its Distribution (River Paiva, Portugal)

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Abstract An important population of the critically endangered pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) was surveyed at the edge of its southern distribution (River Paiva, Portugal). Although an earlier study suggested that this population had a very low number of individuals (<500), a narrow distribution, and was mainly comprised by old specimens our data contradict these findings. Our assessment estimated a population with probably more than 5,000 individuals distributed across 80 km of the river length. From the 32 sites surveyed, 19 contained *M. margaritifera* with higher abundances verified in the middle and upper parts of the river (a maximum of 78 ind. per 100 m of river stretch was recorded). The pearl mussels showed a clear preference for areas near the banks, in shallow water, sandier and gravel sediments, and a high degree of riparian vegetation cover. The population structure was skewed with a very high percentage of large (and old) animals but 3.7 % of the individuals collected were juveniles (<60 mm in length); therefore, this population can be considered functional. Environmental characterization indicated that this river is still in excellent or good condition although some areas showed deterioration due to discharge of domestic effluents. The main conservation requirements of *M. margaritifera* in the River Paiva include maintaining the water quality (and if possible stopping the discharge of domestic effluents), increasing riparian vegetation cover, removing several weirs to increase connectivity, and increasing trout density.

Keywords Conservation · Distribution · Freshwater pearl mussel · *Margaritifera margaritifera* · River Paiva

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Introduction

Freshwater ecosystems are threatened with a myriad of human activities which may ultimately affect biodiversity and ecosystem functions and services (Dudgeon and others 2006; Vörösmarty and others 2010). Main threats such as habitat loss and fragmentation as a result of construction of dams or other obstacles, river bank regularization, pollution, introduction of invasive species, overexploitation of resources, and climate change have been accelerating in the last decades and were responsible for the local or even global extinction of several species (Abell and others 2007; Carpenter and others 2011).

Remarkably, there is a considerable taxonomical bias in relation to groups receiving conservation attention in

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freshwater ecosystems (Ricciardi and Rasmussen 1999). Although mammal and bird species receive a majority of this conservation attention and financial support, these species are not always the most threatened, and overlooked groups such as fish, molluscs, and crustaceans deserve further consideration inside freshwaters (Darwall and others 2011).

Freshwater molluscs have been subjected to high rates of extinction or decline in recent decades (Lydeard and others 2004; Régnier and others 2009) with freshwater mussels (order Unionoida) being especially threatened (Strayer and others 2004). One of the most charismatic species inside this group is the critically endangered *Margaritifera margaritifera* (Linnaeus, 1758), which in recent years has received particular interest, mainly in Europe (Beasley and Roberts 1996; Cosgrove and others 2000; Hastie and others 2000a; Ostrovsky and Popov 2011; Reid and others in press). This interest is probably related with its fascinating life cycle that includes a suitable fish host (*Salmo trutta* or *Salmo salar*) and its requirement for exceptional habitat quality (Bauer 1992; Österling and others 2008, 2010). Historically, this species was widespread, but has suffered massive declines both in distribution and abundance in recent decades. Many populations are facing a high risk of extirpation due to recruitment failure (many populations, although containing adults, completely lack juveniles; Geist 2010; Österling and others 2010). Major threats to this critically endangered species include habitat loss and fragmentation; habitat degradation; decline or disappearance of suitable fish hosts; changes in hydrology, chemistry, or geomorphology of streams; and harvesting by humans (for a review see Geist 2010).

While a great number of studies have been done on *M. margaritifera*, mainly in Northern and Central Europe, populations in the southern part of its distribution have received much less attention (Outeiro and others 2008). Therefore, data about the status of these southern populations are particularly important to follow the possible impact of climate change, for example (Hastie and others 2003). In Portugal, an extensive survey was done by Reis (2003) which recognized the presence of several populations in good condition in the north of the country (Rivers Mente, Rabaçal, and Tuela). This study was particularly relevant because the rediscovery of these populations refuted the idea of the species extinction in Portugal. In addition to the above mentioned rivers, Reis (2003) also found specimens in the Rivers Neiva, Cávado, and Paiva but in these cases only very few individuals were present and mainly comprised adults with no signs of juvenile recruitment. In the last 2 years, additional Portuguese populations were found in the River Tâmega basin (Rivers Beça and Terva; for a full characterization of these populations see Varandas and others 2013).

Considering that freshwater pearl mussels are considered an ideal target species for conservation (this species is usually described as indicator, flagship, keystone, and umbrella species in oligotrophic systems; Geist 2010) it is mandatory that key ecological characteristics (e.g., distribution, abundance, population structure, and characterization of fundamental abiotic conditions) of viable populations are identified. Given the lack of information for *M. margaritifera* populations at the edge of its global southern distribution and considering that this data is vital for its future conservation, the aims of this study were to: (i) survey the *M. margaritifera* population along the entire extension of the River Paiva; (ii) analyze distribution and population structure; (iii) characterize the abiotic conditions, i.e., water quality and habitat (including river banks); and (iv) give an overview of the most pressing threats to this population and propose conservation/restoration measures which could be implemented in future.

Materials and Methods

Study Area

The River Paiva is a tributary of the left margin of the River Douro with a total length of 108 km and a catchment of 795 km^2 entirely located in Portugal. In the headwaters, the river flows through a plateau dominated by areas of agriculture and forestry. In the middle and lower parts, the valley has very steep slopes and is dominated by extensive areas of forestry consisting of mainly pines and eucalyptus, but also with oak and cork. In general this river has well preserved riparian vegetation (e.g., *Alnus glutinosa*) and water quality is excellent. Important mammal species such as *Canis lupus*, *Galemys pyrenaicus*, and *Lutra lutra* are present in the area. Given its ecological and conservational importance this area is classified as a Natura 2000 site.

Sampling Strategy and Data Analysis

Characterization of environmental conditions was carried out in July 2012 at five sites along the entire river gradient (sites S8, S40, S64, S82, and S99; Fig. 1). At each site, the following water column parameters were measured: temperature, dissolved oxygen, pH, conductivity, total suspended solids, nitrites, nitrates, phosphates, calcium, and hardness. The first five environmental factors were measured in situ, close to the bottom, by the use of a multiparametrical sea gage YSI 6820. Nitrites, nitrates, and phosphates were determined by molecular-absorption spectrometry; calcium by flame atomic absorption spectrophotometry; and hardness by complete cation analysis (derived from the calcium and magnesium levels). Samples of benthic macroinvertebrate fauna were collected with a 0.5-mm mesh hand-net, using semi-quantitative techniques over a 50-m long reach. Organisms were obtained by kick-sampling from six transects (1 m long by 0.25 m wide) covering different habitats (inorganic: coarse, sandy, and muddy substrates; organic: algae, aquatic macrophytes, and organic matter; sedimentation and erosion zones) starting at a riffle. Collections were combined and organisms were sorted in the laboratory. Invertebrates were then preserved in 70 % ethanol and identified to the family level. Data obtained were used to calculate the Water Ecological Status (WES) based on a benthic index of biotic integrity (IPtIN—North Invertebrate Portuguese Index; INAG 2009). Physical habitat characterization was done using the River Habitat Survey (RHS) methodology (Raven and others 1998). A principal components analysis (PCA) in the PRIMER 6 package was used to detect differences between sites and was based on the abiotic, WES, and RHS results.

Assessment of the *M. margaritifera* population in the River Paiva was carried out from March to July 2012 over the total length of the river; comprising 32 different sites (see Fig. 1 for site locations). For each site a river stretch of 100 m was visually surveyed using glass bottomed viewers and snorkeling. These surveys were always performed with a minimum of four people spending a minimum of 3 h in each site. For all mussel specimens, geographic coordinates and five instream attributes (overhead cover found immediately around the mussel location, predominant type of riverbed substrate, current velocity, water depth, and distance from the nearest river bank) were recorded to evaluate the mussel habitat preference. 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 the substrate (1 roots; 2 sand and gravel; and 3 cobles, boulders, or bedrock); and six categories for current velocity (qualitative data varying from 0 to 5 where 0 represent a null velocity and 5 a very high velocity). The distance from the river bank, and water depth were measured with a ranging pole and a tape measure, respectively.

Mussel dimensions (shell length, height, and width) were measured to the nearest 0.1 mm with a Vernier caliper. All specimens were carefully returned to the river in their original position after the collection of this information. To infer the population structure and confirm the

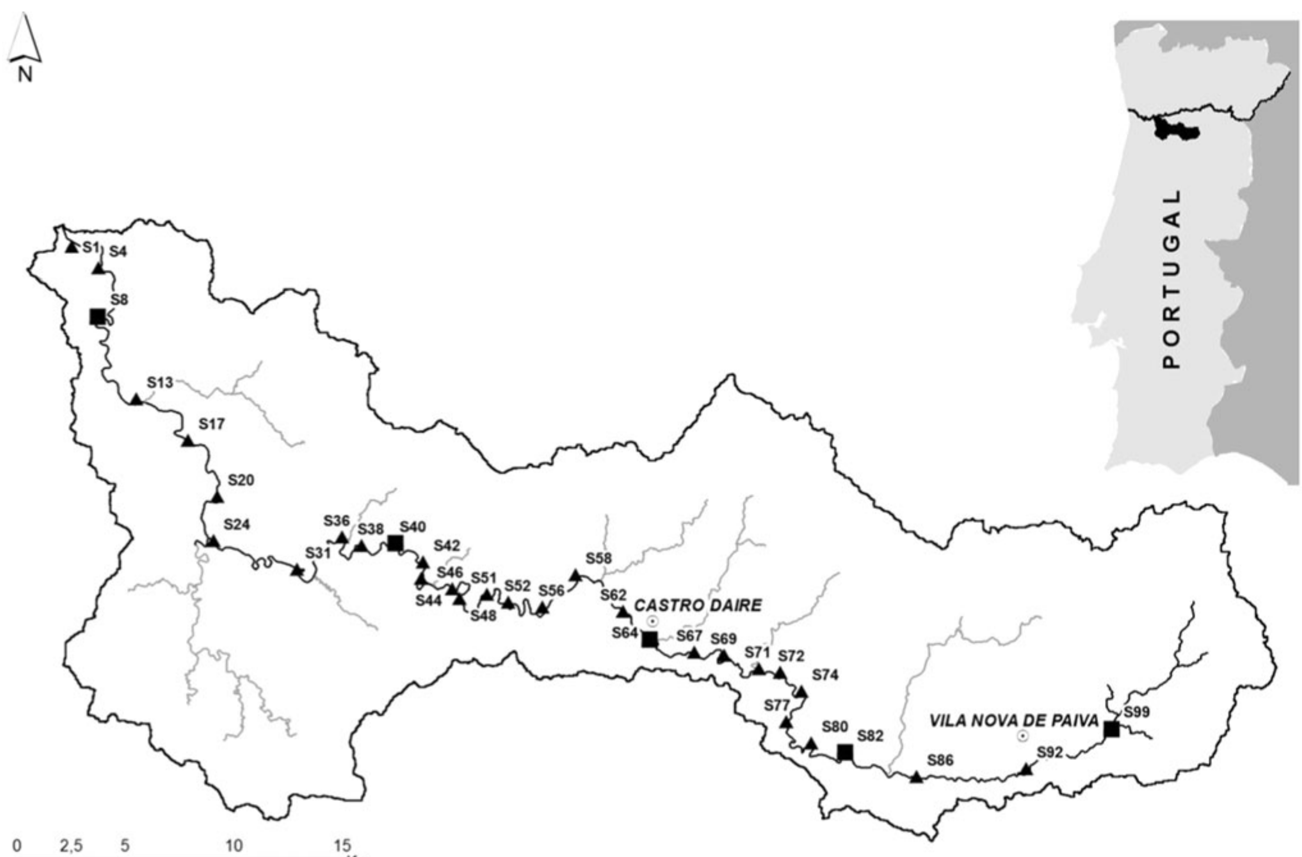


Fig. 1 Map of the River Paiva showing the 32 sites location (sites marked with a quadrate were also sampled for abiotic and fish fauna characterization)

evidence of recent recruitment, a size–frequency distribution using 10 mm intervals was used following Young and others (2001). *M. margaritifera* juveniles were considered using the biologically based definition (up to 60 mm) described by San Miguel and others (2004) for Iberian populations. According to these authors Iberian *M. margaritifera* populations reach maturity much earlier than northern populations, at around 6 years, which implies that specimens larger than 60 mm in length can be considered adults. Possible differences in mean lengths along sites were tested by Kruskal–Wallis since data depart from normality.

Fish fauna was assessed in the same five sites as the environmental characterization using electrofishing (backpack equipment with a pulsed DC-600 V generator). The voltage was set between 150 and 200 V in order to produce a current from 1.5 to 3 A. Stunned fish were placed in containers to recover, identified to species level, counted, and released. The total area surveyed and total numbers of fish captured were recorded and fish densities were expressed as fish per 100 m^2.

Results

Environmental Characterization

Environmental characterization in the five sites along the entire gradient of the River Paiva is presented in Table 1. Overall, there were some differences in the five sites mainly related to temperature that decreased from the mouth (23.5 °C in S8) to the head (19.1 °C in S99) of the river; nitrates had a much higher value at S64; and calcium and hardness had much lower values at S82. A total of 61 macroinvertebrate families were collected from the River Paiva. Aquatic insects belonging to pollution-sensitive orders of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) dominated and the number of EPT families were high (22 families). As a result, WES, assessed using the benthic index of biotic integrity ($IPtI_N$), was considered excellent at S8, S40, and S82 and good in S64 and S99 (Table 1). The evaluation of habitat quality showed that sites in the River Paiva have spatial differences in the RHS scores. Site 8 reached excellent quality and the remaining four sites were classified as good. The PCA (Fig. 2), using the abiotic data plus the results of the WES and RHS, revealed a clear spatial pattern. From this projection, sites appear distributed along an environmental gradient, with downstream sites along one of the edges (sites S8, S40 and S64) and upstream sites (sites S82 and S99) located at the other edge. The main factors responsible for the separation along the first axis were hardness and temperature and along the second axis WES and nitrates.

Margaritifera margaritifera Ecological Status

Margaritifera margaritifera was present in 19 of the 32 sites surveyed (Fig. 3). The most downstream site with individuals was located at S8 and the most upstream at S82 (further upstream no individuals were found although several sites were surveyed). We found a total of 353 individuals being the highest abundance (78 bivalves per 100 m of river stretch) recorded at S71. Several sites have more than 30 individuals per 100 m of the river stretch (S42, S67, S69, and S71) but the majority of sites had low numbers (Fig. 3).

The population structure showed a great dominance of large (and old) specimens (Fig. 4) with a maximum percentage of individuals in the size class of 80–90 mm and more than 80 % of the individuals collected were larger than 70 mm. Only 3.7 % of specimens sampled in the River Paiva could be considered juveniles (lengths lower than 60 mm). The mean mussel size found in the River Paiva was 83.0 mm (±12.9 mm SD). The smallest individual collected was 25.2 mm and the largest was 110.1 mm. There was a clear difference in the mean length of individuals at the different sites ($H = 128.6$; $P < 0.001$; Fig. 5), with the individuals at downstream sites clearly larger than individuals from upstream sites.

More than 70 % of the bivalves were collected within 4 m from the banks, with very few found in the middle of the river channel (Fig. 6). This species also preferentially colonized areas with velocities between categories 1 and 3 (Fig. 6). In relation to depth, the specimens were preferentially found at water depths between 50 and 100 cm and the preferential substrate for *M. margaritifera* was mainly comprised of sand and gravel in areas typically covered by riparian vegetation (Fig. 6).

Fish fauna was comprised of eight different species with one being non-native (*Gobio lozanoi*). Fish densities were very low and dominated by the presence of *Pseudochondrostoma duriense* (comprising 53.8 % of all fishes captured). *S. trutta* was the unique host of *M. margaritifera* in this river and was present at all of the five survey sites but always at very low numbers (Table 2).

Discussion

Almost 80 km of the River Paiva contain *M. margaritifera* individuals and potentially have environmental conditions to support a healthy population (although we were not able to find live specimens between S52 and S67). The absence of specimens upstream of S82 and downstream of S8 may be explained by the lack of suitable habitat conditions for the species and due to lentic conditions caused by a dam in the River Douro which influences the first 4 km of the

Table 1 Environmental characterization along the five sites sampled in the River Paiva

	S8	S40	S64	S82	S99
Temperature (°C)	23.50	22.57	21.43	20.30	19.10
Dissolved oxygen (mg L^{-1})	7.82	8.02	7.80	7.74	7.39
pH	6.45	6.59	6.10	6.00	6.42
Conductivity (μS cm^{-1})	56.50	49.90	54.50	37.50	41.10
Nitrites (mg L^{-1})	<0.03	<0.03	<0.03	<0.03	<0.03
Nitrates (mg L^{-1})	3.11	4.13	7.05	2.35	3.01
Phosphates (mg L^{-1})	<0.31	<0.31	<0.31	<0.31	<0.31
Calcium (mg L^{-1})	1.32	1.32	1.20	0.86	1.20
Hardness (mg L^{-1})	8.60	7.36	6.21	4.78	5.22
Total suspended solids (mg L^{-1})	8.00	10.00	8.00	10.00	7.00
WES ($IPtI_N$ index)	Excellent	Excellent	Good	Excellent	Good
RHS	Excellent	Good	Good	Good	Good

Overall results about the Water Ecological Status (WES) based on macroinvertebrates and the River Habitat Survey (RHS) are also included

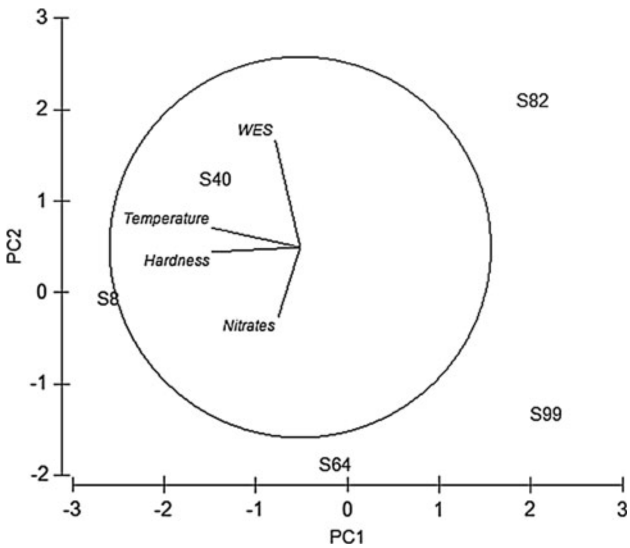
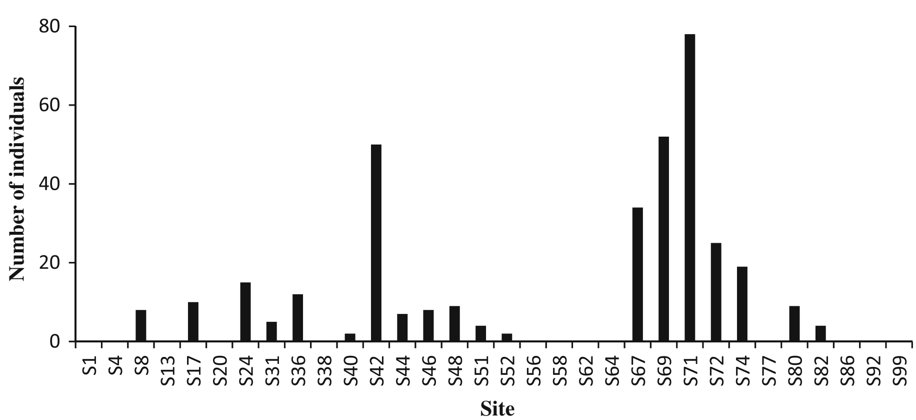


Fig. 2 Principal component analysis (PCA) showing the plotting of the five sites plus the two more informative factors per each axis. The percentage of variability explained by PC1 was 44.3 % and PC2 28.2 %

River Paiva, respectively. On the other hand the absence of specimens from S52 to S67 could be explained by the release of domestic effluents from the village of Castro Daire (site S64). This situation is clearly affecting the water quality since high nitrate levels and a lower WES was measured at site S64 in comparison with the other four sites.

This survey was very intensive and covered 32 different sites. The majority of sites had restricted access which prevented us doing a more complete environmental and fish fauna characterization due to logistic constrains. The abundances reported in this study should be regarded as minimum values due to the possibility of under-estimation of smaller mussels and of buried individuals, which would be hidden and escape our visual assessment. Even so, the total number of *M. margaritifera* may be more than 5,000 individuals along the entire river length. This estimation is based on the extrapolation of mussel abundance at individual sites to the length of the river with suitable habitat. This estimation is much higher than the one made by Reis (2003), which advance with a total population number of

Fig. 3 Number of *M. margaritifera* individuals per 100 m of river stretch along the 32 sites sampled in the River Paiva



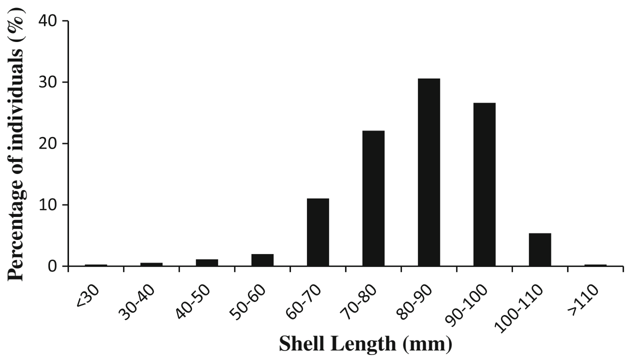


Fig. 4 Population structure of *Margaritifera margaritifera* in the River Paiva ($N = 353$)

fewer than 500 individuals. This difference cannot be explained by a recovery in abundance in such a short period of time between sampling campaigns but by the much higher sampling effort of the present study.

The pearl mussel population structure in the River Paiva reveals acute aging with a great preponderance of individuals measuring over 70 mm in length (more than 80 % of the individuals collected). This situation was already described in many Iberian and other European populations and is the major factor concerning the survival of this species (Álvarez-Claudio and others 2000; Hastie and others 2000b; Morales and others 2004; Outeiro and others 2008; Geist 2010; Österling and others 2010). However, the present study reveals that 3.7 % of specimens sampled can be considered juveniles (lengths lower than 60 mm). Therefore, River Paiva supports a functional pearl mussel population (i.e., at least one juvenile found, regardless of the overall numbers of adults present; Cosgrove and others 2000) mainly in upstream areas (discussed below). In addition, three individuals with a shell length lower than 32 mm were found which means that recent recruitment has occurred in this river. Again, we must keep in mind that our survey was mainly focused in visible animals and so this may considerable under-estimate the presence of small individuals.

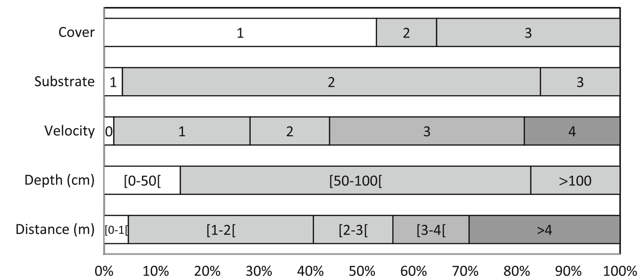
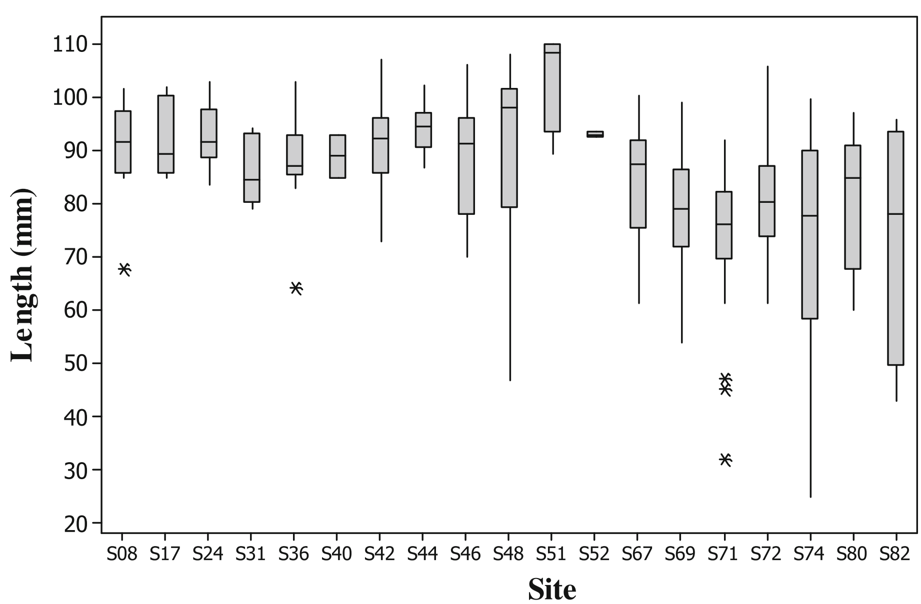


Fig. 6 Percentage of *M. margaritifera* individuals in relation to habitat characteristics: cover (qualitative data being 1 roots or vegetation; 2 cobbles or boulders; and 3 bedrock), dominant substrate (qualitative data being 1 roots; 2 sand and gravel; and 3 cobles, boulders, or bedrock), current velocity (qualitative data varying from 0 to 5 where 0 represent a null velocity and 5 a very high velocity); depth (cm); and distance to banks (m)

Table 2 Density (ind. 100 m^{-2}) of fish species along the five sites sampled in the River Paiva

Species	S8	S40	S64	S82	S99
Achondrostoma oligolepis	–	0.06	0.33	0.57	0.53
Anguilla anguilla	0.32	–	–	–	–
Gobio lozanoi	0.72	–	–	–	–
Luciobarbus bocagei	0.80	0.56	–	–	–
Pseudochondrostoma duriense	2.52	8.72	3.50	1.29	–
Salmo trutta	0.12	0.11	0.17	2.29	0.93
Squalius alburnoides	1.16	1.78	–	–	–
Squalius carolitertii	0.48	0.72	–	1.71	0.40

Fig. 5 Length of *M. margaritifera* in the River Paiva. Boxplots show median values (*central line*), the range from the first to the third quartile (*box*), Turkey whiskers and outliers (*dots*)



In the River Paiva, *M. margaritifera* showed a clear preference for areas near the banks at low depths, sandier and gravel sediments, a high degree of riparian vegetation cover, and in a wide range of current velocities. These preferences have been widely described for other European populations and follow very similar patterns (Álvarez-Claudio and others 2000; Hastie and others 2000b; Morales and others 2004; Outeiro and others 2008). The high abundance in these areas can also be related with the preference of hosts (young trout) (Outeiro and others 2008).

Although the abiotic characterization was restricted to five sites and only covering the summer conditions (and so our conclusions should be considered with some caution), the area downstream S64 seems to present less suitable conditions for *M. margaritifera* mainly due to higher nitrate concentrations. This situation is the more probable explanation for the absence of specimens from S64 to S52 since it is well established that nutrient enrichment may impair the survival of *M. margaritifera*, particularly juveniles (Geist 2010). Since juveniles have specific habitat requirements: cool, well-oxygenated soft water free of pollution or turbidity, the release of domestic effluents may lead to nutrient enrichment and may impair recruitment and mussel survival (Varandas and others 2013). Interestingly, small/young mussels were only found in the area upstream of S64 which corroborates our findings that upstream sites have better environmental quality and are more suitable for mussel recruitment and survival. If our assumptions about nutrient enrichment and low flow conditions in summer adversely affecting *M. margaritifera* are correct, careful management of this situation should be a priority not only for the conservation of *M. margaritifera* but also to guarantee excellent water quality in this river. Thus, if this situation is reversed it is possible that this species could return to the 15-km stretch of the river since suitable physical habitat exists.

The density of *S. trutta* (and also the other seven fish species) was very low along the five sites surveyed and this may be explained by the very low productivity of this river (however, this river has also low conductivity values which in some way can affect the electrofishing efficiency). According to some studies this lower trout density may be problematic for pearl mussels because it may result in lower total glochidia infections and so in a lower or even failure in recruitment (Arvidsson and others 2012). Although some authors suggest a density of at least five juvenile trout per 100 m^2 to sustain a healthy *M. margaritifera* population (Bauer 1988; Ziuganov and others 1994), Geist and others (2006) support that similar densities as reported in the River Paiva may be sufficient to sustain a functional population. According to Geist and others (2006), low densities of host fish can be compensated by the higher glochidia carrying capacity of older host fish with limited previous contact with pearl mussel glochidia, by the long reproductive period of mussels, and by low mortality rates of juvenile mussels during their post-parasitic phase. Although it is impossible to establish a minimum threshold for the required density of host fish, the overall density of trout in the River Paiva seems to be low and this situation should be taken in account in future studies and in the application of conservation measures.

In addition to *M. margaritifera*, we also found three more species of freshwater bivalves in the River Paiva: *Anodonta anatina*, *Corbicula fluminea*, and *Unio delphinus*. The two native species *A. anatina* and *U. delphinus* were found in the first 13 km near the mouth of the river and the invasive *C. fluminea* was found from the mouth to S51. The presence of *C. fluminea* is particularly interesting since this river has very clear waters with oligotrophic conditions and has very high flows in the winter. Even so, this invasive species was able to colonize almost half of the length of this river; although the majority of individuals presented a very poor physiological condition (i.e., individuals with low biomass, shells very eroded and very few individuals had lengths larger than 25 mm). While the abundance of *C. fluminea* in this river was not very high compared to other invaded systems special attention should be given to this species considering the already described ecological and economic impacts (for a review see Sousa and others 2008a, b). Indeed, if environmental conditions changed (increase in productivity due to higher nutrient release from domestic effluents or agricultural areas) it is possible that *C. fluminea* could increase its density and biomass and potentially intensify its ecological impact. In the same vein, the possible interaction between this invasive species and *M. margaritifera* should be taken in account in future studies.

In conclusion, the present work contributes to our knowledge of the current ecological status of *M. margaritifera* at the edge of its southern distribution. This information is important not only in terms of the present conservation status of the species but also as a reference for future alterations, including climate change. Possible alterations due to climate change can be particularly interesting to follow since the River Paiva is near the southern edge of the *M. margaritifera* distribution (in Portugal this river functions as the southern limit of distribution and just two populations in Spain are located further south—Rivers Agueda and Alberche; Velasco and others 2006). In the context of the Iberian Peninsula, the River Paiva can be considered an important habitat for pearl mussels and this population deserves conservational attention and effective protection. Given that the major environmental threats were identified (e.g., nutrient enrichment in medium and downstream areas due to the discharge of domestic effluents, presence of weirs, loss of riparian vegetation, and low density of trout) the main conservation requirements of *M. margaritifera* in River Paiva include maintaining or even improving the water quality (and if possible stopping the discharge of domestic effluents mainly near Castro Daire), increasing riparian vegetation cover, decommissioning several weirs to increase connectivity, and increasing the density of suitable hosts.

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