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Ecology of southern European pearl mussels (Margaritifera margaritifera): *first record of two new populations on the rivers Terva and Beça (Portugal)*

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ABSTRACT

1. Southern European populations of *Margaritifera margaritifera* (L., 1758) are under-studied. From 1986 to 2001 this species was considered extinct in Portugal but between 2001 and 2002 six northern populations were found, five of which were previously unknown.
2. This study comprises a comparative study of the ecology and habitat requirements of two new populations in the rivers Beça and Terva (tributaries of the River Tâmega, northern Portugal) with non-Iberian populations.
3. Surveys were conducted in 2010/2011 to characterize ecological status and propose possible conservation measures. Both rivers were in good environmental condition, but the River Beça had higher biological, physicochemical and hydromorphological quality.
4. Both populations are highly susceptible to extirpation – in particular the River Terva population, given the very low number of specimens found and no sign of recent recruitment. The low number of juveniles and the existence of several threats in both rivers (e.g. fragmentation and loss of habitat caused by the presence of physical obstacles, organic pollution and bank erosion due to fires) imply the need for urgent, effective, conservation measures.
5. Southern European *M. margaritifera* populations have similar ecological and habitat requirements compared with those of northern and central Europe. However, functional populations may endure higher phosphate content, pH and temperature values. As expected they present faster growth rates and reduced life spans.

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KEY WORDS: conservation; distribution; freshwater pearl mussel; *Margaritifera margaritifera;* River Beça; River Terva

INTRODUCTION

The freshwater pearl mussel *Margaritifera margaritifera* (L., 1758) is a widespread Holartic species which is distributed on both sides of the Atlantic (Young *et al*., 2001a, b; Geist, 2010). This species is one of the longest-living invertebrates (Bauer, 1992; Beasley and Roberts, 1999; Österling *et al*., 2008) and was

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until recently one of the most abundant Holarctic freshwater mussel species (Geist, 2010). However, since the 1900s this species has suffered serious decline in several aquatic ecosystems, becoming extirpated in many European regions (Buddensiek, 1995; Frank and Gerstmann, 2007). The global decline of this highly endangered naiad in Europe has caused much concern over the past decades; numerous studies have documented the status of remaining populations and highlighted several causes for their decline (Bauer, 1983; Bauer and Vogel, 1987; Beasley and Roberts, 1996; Álvarez-Claudio *et al*., 2000; Reis, 2003; Frank and Gerstmann, 2007). Although the causes for this catastrophic reduction and local extinctions are not fully understood, they are most likely related to loss, alteration, and degradation of habitat (Österling *et al*., 2008, 2010; Geist, 2010). Over-harvesting for pearls dating back to Roman times (Young and Williams, 1983), together with water pollution and eutrophication, river regulation, drainage, dredging, introduction of exotic fish species, and decreases in host fish species have also been implicated in the decline of freshwater pearl mussel populations (Young and Williams, 1983; Bauer, 1988).

Given the marked decline in recent decades, *M. margaritifera* is internationally protected by the Bern Convention (Annex III) and the EC Habitats Directive (Annex II and V). It has also been listed as 'endangered' in the IUCN Red List of Threatened Species, and 'critically endangered' in Europe (Cuttelod *et al*., 2011; IUCN, 2011).

Historically, the first descriptive report on *M. margaritifera* in Portugal was based on a single shell collected by Morelet in 1845, from the River Tâmega, who described it as a new species (*Unio tristis*). Data published by Nobre (1941) confirmed the presence of pearl mussels in Portugal. Nobre wrote that *M. margaritifera* occurred all over the country; in the River Douro and some tributaries (rivers Tâmega, Sousa, Paiva and Ferreira) in the north, the River Vouga basin in central Portugal, and in the River Mira basin in the south. The last of these records was probably a misidentification since the habitat characteristics and fish fauna of this region do not correspond to the requirements of this species. Bauer (1986) visited the Nobre sites in the River Douro basin and could not confirm any trace of *M. margaritifera*. Later, Young *et al*. (2001a, b) stated that the pearl mussel was extinct in Portugal. However, Reis (2003, 2006) described the presence of freshwater pearl mussels in six rivers (Rabaçal, Mente, Tuela and Paiva in the River Douro basin and in the rivers Cávado and Neiva) located in northern Portugal. Southern populations of *M. margaritifera* are relatively poorly studied and there is a great need for sound basic information on ecology and conservation.

This study compares the data from southern, western, and northern populations and includes data on two newly discovered freshwater pearl mussel populations in two tributaries of the River Tâmega – the rivers Beça and Terva in northern Portugal. Therefore, the main aims of this study were to: (i) compare the ecology and conservation status of southern European populations with previously published results mainly from the northern and western European populations; (ii) determine the density and size distribution in the two recently discovered populations; (iii) assess the population age-structure and its status (viability); (iv) determine the availability of fish hosts; (v) describe the major habitat characteristics and assess the water quality; and (vi) identify the main threats in both rivers and propose conservation measures that could be applied in the near future.

MATERIAL AND METHODS

Study area

The river basins of the River Beça (area 337 km^2, 46 km length) and the River Terva (area 101 km^2, 27 km length) (Figure 1) are characterized in upstream areas by unproductive soils with unimproved vegetation. In the middle reaches, the main water uses are irrigation of natural grasslands (water meadows) and agricultural fields. Lower reaches (below an altitude of 750 m) are heavily forested with pines (*Pinus pinaster*). Well-preserved riparian vegetation is still present and is mostly dominated by *Fraxinus angustifolia*, *Alnus glutinosa*, *Salix atrocinera*, and *Betula pubescens*.

The River Beça basin (mean annual rainfall 1640 mm, 50 year database) has generally good environmental conditions owing to the low levels of human pressure. However, the presence of several weirs for irrigation and hydropower dams result in loss of connectivity and flow regulation. The River Terva has higher levels of human pressure (urban and agriculture) in upstream areas compared with the River Beça, resulting in higher nutrient loads that compromise water quality, in particular during the summer. These conditions are compounded by lower precipitation levels (annual rainfall 700–800 mm) and the presence of

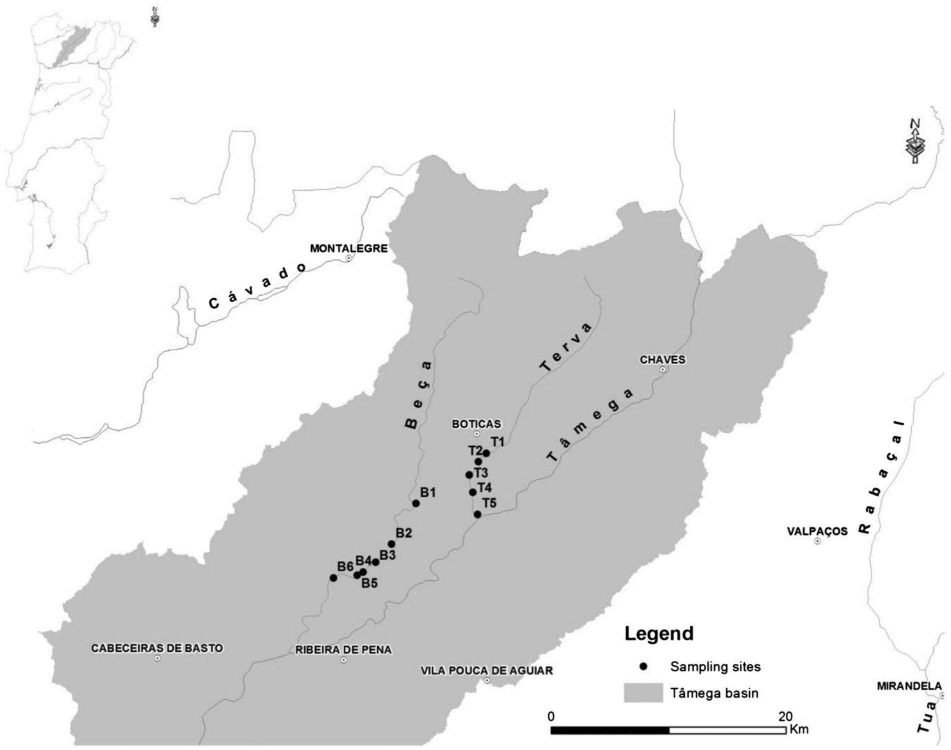

Figure 1. Map of the study area showing the sampling sites (river reaches) in the rivers Beça and Terva.

weirs and dams along the river. In addition, an Inter-Municipal Solid Waste plant facility releases effluent into the lower third of the river. The study area has been affected by severe wildfires over the last decade that may have resulted in high sediment inputs to both rivers with potentially adverse effects on aquatic biota.

Habitat characterization and water quality

To assess the ecological status of both rivers, data on physicochemical, biological, and hydromorphological elements were obtained. During summer base-flow conditions the following *in situ* physicochemical parameters were measured monthly between June and August 2011 at two sites in both rivers using portable meters: water temperature (°C), pH, dissolved oxygen (mg O_2 L^{-1}), and specific conductance (μS cm^{-1}). Water samples were also collected, kept in coolers and transported to the laboratory for analyses using standard methods. The parameters analysed were acid neutralizing capacity (mg HCO_3^- L^{-1}), nitrate (mg NO_3^- L^{-1}), ammonia (mg NH_4^+ L^{-1}), orthophosphate (mg P L^{-1}), biochemical oxygen demand (BOD_5 – mg O_2 L^{-1}), chemical oxygen demand (COD – mg O_2 L^{-1}), and total suspended solids (TSS – mg L^{-1}). Values were classified according to the criteria of the Portuguese Water Institute (INAG). This classification comprises five different classes and a site is considered unimpaired if it belongs to class A and impaired if it belongs to classes D or E.

Samples of benthic macroinvertebrate fauna were collected with a 500 μm 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 – CPOM; sedimentation and erosion zones) starting at a riffle. Collections were combined and organisms were sorted alive in the laboratory. Invertebrates were preserved in 70% ethanol and identified to the family level. Data obtained were used to calculate a benthic index of biotic integrity ($IPtI_N$ – North Invertebrate Portuguese Index; INAG, 2009). The final $IPtI_N$ normalized value was expressed as an Ecological Quality Ratio (EQR, numerical value scale between 0 and 1), where 1 represents (type-specific) reference conditions and values close to 0 indicate bad ecological status. Cutoff values used to classify the studied rivers of northern Portugal are presented in Table 1.

Physical characterization of the habitat at all sites where *M. margaritifera* occurred was obtained by using the River Habitat Survey methodology

Table 1. Water ecological status according to $IPtI_N$ EQR values (INAG, 2009).

River	Typology	$IPtI_N$ Reference value	$IPtI_N$ EQR values	Ecological status
Beça	Mid-large dimension rivers of northern Portugal (N1 > 100 km^2)	1.00	>0.87	High
			0.86 – 0.65	Good
			0.64 – 0.44	Moderate
			0.43 – 0.22	Poor
			< 0.22	Bad
Terva	Small dimension rivers of northern Portugal (N1 ≤ 100 km^2)	1.02	>0.88	High
			0.87 – 0.66	Good
			0.65 – 0.44	Moderate
			0.43 – 0.22	Poor
			< 0.22	Bad

(RHS – Raven *et al.*, 1997, 1998). RHS assesses habitat quality over a 500 m reach and within a 50 m buffer on each bank. Hydromorphological river quality was expressed via the Habitat Quality Assessment (HQA, version 2.1) and Habitat Modification Score (HMS, version 2003) indices, calculated from RHS survey information. The software RAPID 2.1 was used (http://www.ceh.ac.uk/products/software/RAPID.html) for data input and storage of the field data, and to calculate HQA and HMS.

Distribution and density of *M. margaritifera*

Surveys were carried out between 2010 and 2011 over the last 17 km and 10 km of the rivers Terva and Beça, respectively. In total, 11 sites were surveyed (six in the River Beça and five in the River Terva); at each site a minimum of 250 m and a maximum of 1750 m were thoroughly checked for the presence of freshwater pearl mussels (see Figure 1 for site locations).

Only the visible individuals were sampled using bathyscopes (glass-bottomed viewers) and snorkeling. These surveys were always performed with a minimum of four persons and with a minimum of 4 h spent at each site. For all mussel specimens, geographic coordinates and five instream attributes (overhead cover found immediately around the mussel location, predominant type of river-bed substrate, mean current velocity, mean water column 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: (a) seven categories for cover (absent; cobble/stones; boulders/bedrock; overhanging vegetation; submerged roots and overhanging vegetation; stones, boulders, and overhanging vegetation; undercut bank); and (b) four categories for the substrate (fine sand/gravel; pebble/cobble; stones; boulder/bedrock) and current velocity (no flow to very low flow; low flow; moderate flow; high flow). The distance from the river bank, and water depth were measured with a ranging pole and a tape measure, respectively.

The length and wetted area of each reach were measured in the field and the total length of river sections was calculated using GIS (Table 2). Mussel densities were determined based on the total area surveyed in each reach and the same method was used to estimate the total number in each river.

Table 2. Number of individuals and average density of freshwater pearl mussel in the reaches studied in the rivers Beça and Terva

River reaches	Reach length (m)	Reach width (m)	Reach area (m^2)	Number of mussels	Mussel density (mussels m^{-2})	Total river population (estimate)
Beça 1	300	6	1800	0	0.000	<600
Beça 2	600	12.5	7500	1	0.0001	
Beça 3	950	8.5	8075	1	0.0001	
Beça 4	250	9.5	2375	6	0.003	
Beça 5	750	10	7500	76	0.010	
Beça 6	750	14	9750	99	0.010	
Length of River Beça inhabited (km)				10		
Terva 1	900	12	10800	0	0.000	< 20
Terva 2	1000	8	8000	14	0.002	
Terva 3	800	7	5600	0	0.000	
Terva 4	1750	8	14000	0	0.000	
Terva 5	800	9	7200	0	0.000	
Length of River Terva inhabited (km)				1		

Age and population structure

Mussel dimensions (shell length, height, and width) were measured to the nearest 0.01 mm with a Vernier caliper. All specimens were carefully returned to the river in their original position after collecting information. Age structure of each population was evaluated based on growth parameters, derived from von Bertalanffy's equation, established for freshwater pearl mussel populations in north-western Spain (San Miguel *et al.*, 2004). Parameters for the River Landro were used because it was the site where the highest maximum shell length value (Lmax) was observed, allowing ages for a greater number of individuals to be calculated.

To infer the population structure and confirm evidence of recent recruitment, a size–frequency distribution using 5 mm intervals was used following Young *et al.* (2001a, b). *Margaritifera margaritifera* juveniles were defined as those up to 60 mm, described by San Miguel *et al.* (2004) for Iberian populations.

According to these authors, Iberian *M. margaritifera* populations reach maturity much earlier than northern populations (at *ca* 6 yr) which implies that specimens larger than 60 mm can be considered adults.

Host populations

Brown trout densities (host fish population) were estimated at the mussel sites using electrofishing (backpack equipment with a pulsed DC-600V generator) to assess the occurrence and density of suitable fish for mussel recruitment. The voltage was set between 150 and 200 V in order to produce a current from 1.5 to 3 A. Electrofishing was carried out in both rivers during May 2011 using a single pass and following a CPUE approach (constant capture effort in each meso-habitat) in all the habitats (100 m, total distance surveyed). Stunned fish were placed in containers to recover, identified to species level, counted, and released.

RESULTS

Water quality and habitat characterization

Physicochemical water quality data from the two rivers are presented in Table 3. The rivers studied differed considerably in most of the parameters analysed, except dissolved oxygen (9.2–9.5 mg O_2 L^{-1}) and pH (6.6–6.8). The average water temperature ranged between 13.2°C in the River Beça and 15.9°C in the River Terva. The River Terva had moderately high conductivities with mean values up to 96 µS cm^{-1} at Terva 1 and Terva 2. This value was 3.3 times higher than those obtained for the River Beça (Table 3). Data on water chemistry also showed that the remaining parameters evaluated (BOD, COD, TSS, nitrate, ammonia, and orthophosphate) were higher in the River Terva. However, these values indicated good quality class (class B). It must be emphasized that all values obtained for the River Beça were considerably lower than those for the River Terva, which suggests a river in excellent condition (class A).

In total, 38 macroinvertebrate families (comprising 13 different classes/orders) were collected from the River Terva and 32 (distributed in 11 classes/orders) from the River Beça. Aquatic insects belonging to pollution-sensitive orders of Ephemeroptera

Table 3. Water physico-chemical parameters (mean, min, and max values) of the rivers Beça and Terva in 2011. Values measured between June and August at two sites in both rivers

	River Beça			River Terva		
	min	mean	max	min	mean	max
Temperature (°C)	11.2	13.2	14.8	15.3	15.9	16.5
Conductivity (µS cm^{-1})	27.1	29.4	31.6	87.2	96.0	107.8
pH	6.4	6.6	6.8	6.6	6.8	6.9
O_2 (mg L^{-1})	9.0	9.5	10.1	8.9	9.2	9.7
O_2 (%)	92.2	96.7	101.2	89.9	91.8	95.6
BOD_5 (mg O_2 L^{-1})	1.2	1.4	1.5	2.7	3.5	4.4
Nitrate (mg NO_3^- L^{-1})	<2	<2	<2	22	24	25.9
Ammonium (mg NH_4^+ L^{-1})	0.04	0.05	0.05	0,86	0.98	1.1
Orthophosphate (mg P L^{-1})	0.02	0.04	0.04	0.13	0.13	0.14
Alkalinity (mg HCO_3^- L^{-1})	14.9	15.3	15.5	22.8	25.0	27.9
COD (mg O_2 L^{-1})	8.5	10.1	11.0	14.0	20.3	24.6
TSS (mg L^{-1})	17.9	19.0	20.0	24.7	28.2	32.7

(mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) dominated in both rivers (Terva with 71.5% and Beça with 68.0%). The number of EPT families was very high (14 and 16 families, respectively, for the rivers Beça and Terva), exceeding (for the River Beça), the reference value (13) for mid–large rivers in northern Portugal. The most tolerant taxa (Gastropoda, Oligochaeta, and Hirudinea) were seldom found in both rivers (0.9% for the River Beça and 6.9% for River Terva). As a result, water quality, assessed using the benthic index of biotic integrity ($IPtI_N$), was considered excellent in both rivers (River Terva EQR = 0.94; River Beça EQR =1.06).

RHS showed that sites in the River Terva have spatial differences in the HMS scores. Terva 1 and Terva 2 were the worst quality (class 5 and 4, respectively) while the remaining sites ranged from obviously modified (Terva 3 and 4) to good quality (Terva 5). In contrast, all sites in the River Beça were obviously modified (class 3). This classification indicates anthropogenic modifications such as bank reinforcement, modified bank profile, and presence of weirs. Despite the low HMS scores recorded, the HQA index exhibited excellent quality (class 5) along all sites in both rivers.

Distribution and density of *M. margaritifera*

Mussels were absent from several surveyed sites; the maximum inhabited length was 10 km and 1 km in the rivers Beça and Terva, respectively (Table 2). In the River Beça, *M. margaritifera* was found at five of the six sampled areas (the exception was Beça 1), and in the Terva it was present at just one site (Terva 2).

Estimated densities were low in both rivers but higher in the River Beça (mean values ranging from 0 to 0.01 ind m^{-2}) than in the River Terva (mean values ranging from 0 to 0.002 ind m^{-2}) (Table 2). The highest densities of pearl mussels occurred in the two downstream reaches of the River Beça (Beça 5 and Beça 6). A population of 520 individuals was estimated for the River Beça, but only 14 living pearl mussels were found in the River Terva. However, at Terva 4 28 well preserved shells were found on the river banks (comprising a total area of 930 m^2) which ranged between 68 mm and 98 mm. No dead shells were found at the remaining sites.

In the River Beça, mussels were distributed at all flow velocities, distances from river banks, substrate and cover types, and from depths ranging between 0.2 and 2.5 m (Figure 2). Small clumps of mussels (2–12 individuals) also occurred on the river bed mainly in shallow waters (no more than 0.6 m depth) with low flows and protected by boulders/bedrock (>30 cm) and riparian vegetation cover. Overall, *M. margaritifera* was generally found near shady river banks (distance from the nearest bank less than 2 m – 70.7%, and sheltered among the stones, boulders or submerged tree roots and covered with overhanging vegetation – 67%) and shallow water at depths less than 0.8 m (total of 91.8%: 39.3% between 0.2 and 0.4 m, 31.7% between 0.4 and 0.5 m and 20.8% between 0.6 and 0.8 m) (Figure 2(a), 2(b), and 2(c)). Mussel densities were greatest in the sand/gravel dominant substrate (50.8%) compared with stones and pebble/cobble (total of 38.3%: 19.1% respectively for each substrate) and boulder/bedrock substrate (10.9%) (Figure 2(d)). The highest densities of freshwater pearl mussel were mostly in running waters with slow flow (total of 85.3% in categories 1 and 2) (Figure 2(e)). No dead shells of *M. margaritifera* were found at the six sampling sites.

Despite the small number of mussels found in the River Terva, specimens apparently prefer moderate to high flows and were partially buried and protected by boulders or in beds with mixed substrates of cobble, stones, and coarse sand (data not shown). Bivalves found in mixed substrates had a layer of filamentous green algae attached to the periostracum and were surrounded by rooted macrophytes.

Age and population structure

Size–frequency histograms for the rivers Beça and Terva (Figure 3) reveal unimodal distributions for both rivers with a distinct absence of small individuals in the River Terva (Figures 3 and 4). In the River Terva, all living specimens collected had lengths greater than 88 mm (mean of 105.0 mm $\pm$ 8.8 mm standard deviation) with no signs of recent recruitment. The average mussel size found in the River Beça was 94 mm ($\pm$21.3 mm). In both rivers the population structure was skewed towards larger sizes (maximum frequency was in the size class of 110–115 mm) but the River Beça had a much higher number of juveniles (Figure 3). The maximum observed shell length (Lmax) values were distinct in both populations (123 mm in Beça and 114 mm in Terva) and also among sampling sites in the River Beça (from 90 mm in Beça 3 to 123 mm in Beça 6; Table 4). A relatively large number of juveniles (<60 mm) were found in Beça

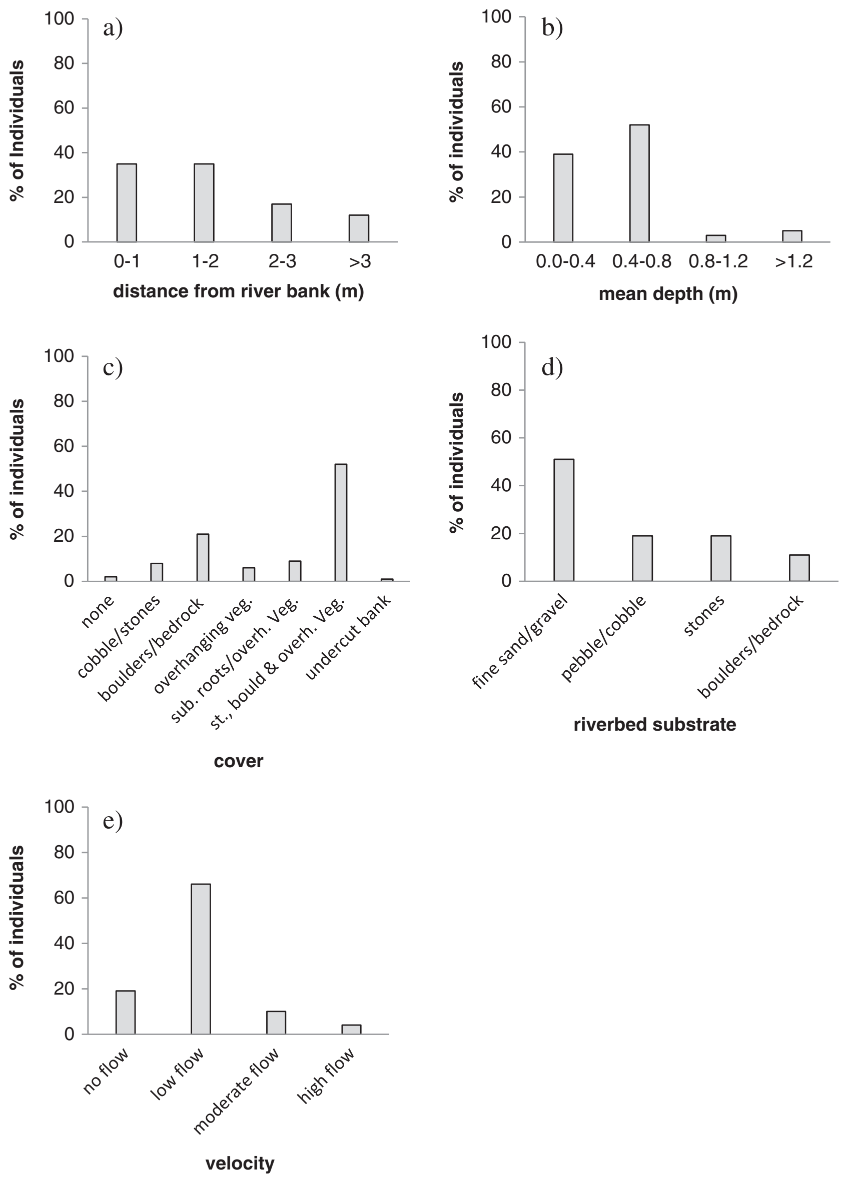

Figure 2. Level of habitat utilization of mussels in the River Beça for: (a) different distances from the nearest river bank observed; (b) mean depth observed; (c) cover type; (d) river-bed substrate (predominant type); and (e) mean current velocity.

5 (25% of all individuals collected at this site) and this was also the site with the highest density of pearl mussels. A very small number of juveniles were also observed in Beça 6 (1%). The youngest live mussel was found in Beça 5 and was 4 years old (shell about 29 mm long). The oldest was also found in Beça 6 with an estimated age ≥ 50 years (123 mm long) (Table 4).

Host populations

Five and six fish species belonging to two and three families were recorded for the rivers Beça and Terva, respectively. Cyprinidae varied between 52 and 71% of all captures for the Beça and Terva, respectively, and was represented by four Iberian endemic species: *Pseudochondrostoma duriense*, *Luciobarbus bocagei*, *Squalius carolitertii* and *Squalius alburnoides*. Density in this family varied between 0.124 and 0.208 ind m^{-2} for the rivers Beça and Terva, respectively. Salmonidae (comprising only the native species *Salmo trutta fario*) represented the rest of the captures in both rivers. This single species, which represents the host of *M. margaritifera*, was the most abundant species (0.116 ind.m^{-2}, comprising 48% of all individuals captured) in the River Beça and the second most abundant species in the River Terva (0.208 ind m^{-2}, comprising 29% of all individuals captured). The invasive *Lepomis gibbosus* (Centrarchidae) was

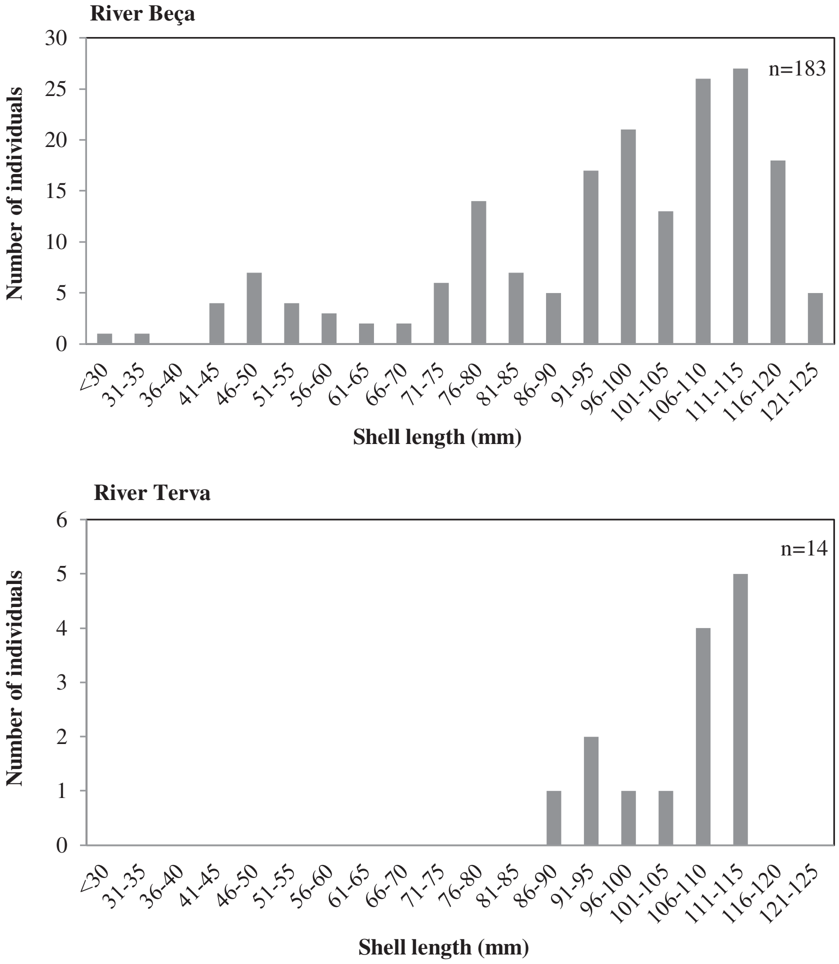

Figure 3. Length profiles of *M. margaritifera* populations from the rivers Beça and Terva

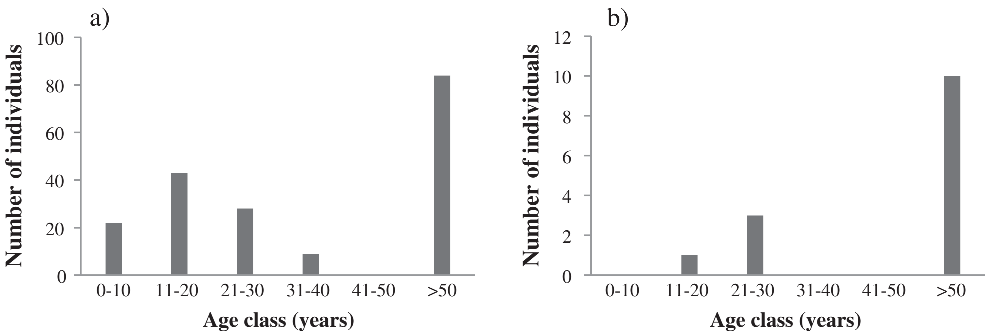

Figure 4. Age-frequency distribution of *M. margaritifera* in the rivers Beça (a) and Terva (b), given for 10-year age intervals

found only in the River Terva, comprising less than 1% of all the individuals captured.

DISCUSSION

Freshwater pearl mussel *M. margaritifera* was probably very abundant in the north of Portugal until the first half of the 20th century, mainly in the Douro and Vouga hydrological basins (Nobre, 1941). Since then, the quality of most of these rivers has deteriorated resulting in the drastic decline or disappearance of this species. The same situation has been described in many European countries (Bauer, 1986; Young *et al*., 2001; Reis 2003, 2006; Geist, 2010; Hastie *et al*., 2010). In Portugal, pearl mussels are still present but only in

Table 4. Observed maximum and minimum shell lengths (Lmax) and ages (Amax) for Beça and Terva *Margaritifera margaritifera* populations by using the estimated parameters for the River Landro (based on San Miguel *et al.*, 2004)

River reaches	N	L_{min} (mm)	L_{max} (mm)	A_{min} (years)	A_{max} (years)
Beça 1	0	--	--	--	--
Beça 2	1	111.0	111.0	> 50	> 50
Beça 3	1	90.0	90	19	19
Beça 4	6	74.6	100.4	12	40
Beça 5	76	29.0	121.9	4	> 50
Beça 6	72	50.4	123.0	7	> 50
Terva 1	0	--	--	--	--
Terva 2	14	88.0	114.0	18	> 50
Terva 3	0	--	--	--	--
Terva 4	0	--	--	--	--
Terva 5	0	--	--	--	--

the upper reaches of river systems that are less impaired by human activities. This study describes the discovery of two more populations (rivers Beça and Terva) to the six recently described by Reis (2003).

The results show that *M. margaritifera* occurred typically in reaches with low depths, relatively low velocities and medium-sized substrate. It was also mainly found in habitats protected by boulders, bedrock, and riparian vegetation. These results concur with others showing that similar substrate (Ostrovsky and Popov, 2011), depths, and velocities (Hastie *et al.*, 2000c; Ostrovsky and Popov, 2011) were optimal habitat characteristics for *M. margaritifera*. Skinner *et al.* (2003) also found that mussels in many rivers of England and Wales were associated with shaded areas created by overhanging herbaceous vegetation and/or scrub and bank-side trees. In two Galician rivers (Eo and Masma, north-west Spain) Outeiro *et al.* (2008) found that pearl mussels had a preference for the strip of river bed within 1.5 m from the river bank, and verified that *M. margaritifera* also inhabited sites with more than 80% tree cover, avoiding sites with less than 50%. However, these findings may depend on the context and some caution is necessary since this study (and other published studies) was on mussel habitats in already depleted populations. According to Moorkens (2000, 2010) mussels in depleted populations where nutrient enrichment is a problem are found close to banks and in the shade of overhanging trees. However, in large populations in oligotrophic conditions, mussels spread out across the river bed and colonize open areas.

Freshwater pearl mussels, in particular the juveniles, have specific habitat requirements: cool, well-oxygenated soft water free of pollution or turbidity. Individuals burrow into sandy substrates, often between boulders and pebbles, in fast-flowing rivers and streams (Hastie *et al.*, 2003b; Geist and Auerswald, 2007). The results of this study show that some of these features do not occur in the River Terva but are more consistent with conditions in the River Beça, which has excellent physico-chemical quality. The decline of water quality, mainly in the upper reaches of the River Terva, is the result of urban and agricultural pressure intensified by lower precipitation and the release of effluent by the Inter-Municipal Solid Waste plant facility. This causes nutrient enrichment of its waters (accompanied by high values of conductivity, BOD, COD, TSS, nitrate, ammonia, and orthophosphate) which leads to an increase in macrophyte growth, covering 40% or more of the river bed from mid-spring to mid-autumn. This high primary production results in a large mass of vegetation that may lower the oxygen levels in the sediment impairing colonization by freshwater pearl mussels (Degerman *et al.*, 2009). According to Beasley and Roberts (1996) nutrient enrichment resulting from agriculture and sewage disposal represents a serious threat to pearl mussels. In addition, Costello *et al.* (1998) found direct evidence of fouling due to cattle wastes and application of agricultural fertilizer, regarding these sources as major threats to *M. margaritifera* in the River Nore (Ireland).

Overall, hydromorphological river quality, expressed by the HQA and HMS indices calculated from RHS survey data, deviated considerably from the reference values showing that habitat quality may be an important influence on the ecological status of the rivers Beça and Terva. Although both rivers have many weirs and dams along the watercourses affecting the natural flow, the River Terva is the most affected especially during periods of low precipitation. This situation drastically reduces flow during the summer and, consequently, there are more areas with standing water and increased sedimentation rates. In addition, the risk of some reaches drying up during the summer is higher. Many authors (Richter *et al.*, 1997; Wilcove *et al.*, 1998; Cosgrove *et al.*, 2000) claim that habitat degradation is one of the major reasons for population declines and species extinctions. Indeed, even with good water quality a healthy aquatic ecosystem cannot be supported if suitable habitat is not present (Metzeling *et al.*, 2004).

Combined information on physical characteristics and water quality indicates that the River Beça has the ability to support a healthier aquatic community owing to the absence of significant threats in the 10 km reach where the mussels occur. This area is near-natural with dense riparian vegetation. This finding is consistent with Moorkens (2010) who verified an association between the best mussel areas and low-intensity land use. The pearl mussel population in this reach was estimated at more than 500 individuals. Although only 20 mussels less than 6 years old (60 mm) were found (10.9% of the population in approximately 4 km) at Beça 5 and Beça 6, the population could be classified as 'functional' according to Cosgrove *et al*. (2000). It is possible that the sampling techniques used in this survey (only visible mussels were sampled) may considerably underestimate the presence of the youngest age classes because many individuals can be buried (Hastie and Cosgrove, 2002; Hastie, 2006; Hastie *et al*., 2010). In the River Terva all living mussels were more than 17 years old and no mussels under the age of 10 were found. Given these results, *M. margaritifera* in the River Terva seems to be almost extinct since there is no evidence of recent recruitment (all specimens were more than 88 mm in length) and the number of individuals found is very low. It is interesting that in an earlier study (Sousa *et al*., 2012) 28 dead shells were found as a result of deaths that occurred during the 2009/2010 severe winter. This finding could be interpreted in two ways: (i) the population in the River Terva supports many more than the 14 individuals found in the present study but the patches with these higher densities could not be found; and (ii) the severe 2009/2010 winter resulted in a significant reduction in the population, which will lead to its extirpation in the River Terva.

A comparison of the results of the habitat requirements and characteristics of the functional Beça population with others from populations in southern Europe and northern and central Europe (Appendix 1) show that the habitat requirements of *M. margaritifera* are similar in almost all areas. These include the substrate, riparian habitat and nutrient-poor water, although phosphate, pH and temperature values are much higher in southern Europe than in non-Iberian populations. In addition, Portuguese populations seem to prefer lower stream velocities compared with other populations in Europe (Appendix 1) where flow preferences are extremely variable and where pearl mussels may frequently be found in fast flows. As expected, increased temperatures increase metabolic rates close to the physiological (and reproductive) limits of the species, resulting in higher growth rates and a lower maximum age. As a result, the Beça population exhibited the highest observed maximum length known for *M. margaritifera* to date in the Iberian Peninsula, exceeding the River Landro population (Spain) with an Lmax of 117.32 mm (San Miguel *et al*., 2004). This phenomenon had been observed previously by Reis (2003) and Bauer (1991) who found latitudinal variation in the longevity of freshwater pearl mussels indicating that populations in southern rivers have a shorter life span. However, some caution is necessary in the interpretation of these results since we used data collected earlier in the River Landro, located more than 200 km north of the rivers Beça and Terva.

Conservation measures

Although it is almost impossible to prevent the extinction trajectory for the River Terva population of *M. margaritifera*, some hope still exists for the River Beça population if urgent conservation measures are applied such as:

- restoration of the 10 km of pearl mussel habitat by replanting and increasing riparian vegetation where it has disappeared due to wildfires. This vegetation provides shelter for trout, augments habitat complexity, maintains low temperatures and well-oxygenated waters during summer, and moderates flow. The installation of bankside fencing would aid vegetation recovery and prevent bank erosion by excluding livestock.
- maintainance or improvement of water quality by creating buffer strips between the river and agricultural land to reduce sediment and nutrient run-off. Provide alternative watering supplies for cattle, preventing bank erosion by trampling, and improving bank stability. These structures would help to decrease erosion, preventing siltation and limiting their detrimental impacts on pearl mussels and host fish.
- logging should be banned along the banks.
- the small hydropower dam must ensure adequate environmental flow releases.
- removal of all the physical obstacles (obsolete weirs) along the 10 km inhabited by *M. margaritifera*, in order to improve longitudinal connectivity (migration of host fish) and to reduce siltation.
- expansion of burned pine areas with deciduous trees (mainly native *Quercus* spp.), usually confined to thalweg lines, creating firebreaks.
- increase of the control and surveillance of fishing activity.
- engagement of local citizens in applied conservation measures for securing survival of pearl mussel populations.

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APPENDIX 1

Bibliographic data collected on: (A) habitat requirements of *Margaritifera margaritifera*; (B) characteristics of functional *Margaritifera margaritifera* populations

A - Ecological requirements		Region	References
Altitude (m)	- 500-700	Austria	Moog *et al*., 1998
	- 50-100	Ireland	Wilson *et al*., 2011
	- 200-700	Portugal	Teixeira *et al*., 2010
	- 400-500	Portugal	This Study
Depth (m)	- 0.5-2	Russia / Sweden	Ziuganov *et al*., 1994; Hendelberg, 1961
	- 0.2-0.6	Russia	Ostrovsky and Popov. 2011
	- maximum depths 1-1.4	Northern and Central Europe	Boycott, 1936
	- 1-3 to prevent freezing	Finland	Valovirta, 1995
	- optimum: 0.3-0.4	Scotland	Hastie *et al*., 2000a
	- optimum: 0.2	Ireland	Gittings *et al*., 1998
	- 0.5-1.5	Germany	Buddensiek *et al*., 1993

(*Continues*)

Table 1. Continued

A - Ecological requirements		**Region**	**References**
	- <1	Spain	Ocharan *et al*., 1997; Álvarez-Claudio *et al*., 2000
	- <0.4	Spain	Morales *et a*l., 2004
	- <2	Portugal	Reis, 2003
	- optimum: 0.3-0.4	Portugal	Teixeira *et al*., 2010
	- <0.8	Portugal	This Study
Flow conditions	- 0.3-0.5 m s^{-1}	Russia	Ostrovsky and Popov, 2011
	- 0.1-1.9 m s^{-1}	Sweden	Björk, 1962
	- fast-flowing waters	Northern and Central Europe	Boycott, 1936; Buddensiek *et al*., 1993; Hastie *et al*., 2003a,2003b;
		UK	Skinner *et al*., 2003
	- optimum: 0.25-0.75 m s^{-1}	Scotland	Hastie *et al*., 2000a
	- fast flowing 0.2-0.4 m s^{-1}	Austria	Moog *et al*., 1998
	- mean flow velocity ≤1 m s^{-1}	Spain	Rosas *et al*., 1992; Álvarez-Claudio *et al*., 2000; Outeiro *et al*. 2008
	- clear flowing water (moderate flow often below rapids)	Portugal	Reis, 2003
	- mean flow velocity ≤0.2 m s^{-1}		
	- low flow	Portugal	Teixeira *et al*., 2010
		Portugal	This Study
Water quality	- unpolluted rivers and streams	England	Skinner *et al*., 2003
Tubidity (NTU)	- 0.39-1.4	Sweden	Österling *et al*., 2008
BOD (mg O_2.L^{-1})	- <1.3	England	Oliver, 2000
	- ≤3	Ireland	Moorkens, 2000
	- <1.4	Central Europe	Bauer, 1988
	- <1.5	Portugal	This Study
Dissolved oxygen	- 90-110%	England	Oliver, 2000
	- 100%sat; >9 mg O_2 L^{-1}	Ireland / Austria	Moorkens, 2000
	- 90-110%	Portugal	Reis, 2003
	- >10 mg O_2 L^{-1}	Portugal	Teixeira *et al*., 2010
	- >9 mg O_2 L^{-1}	Portugal	This Study
Temperature	- 0-23 °C	Austria	Moog *et al*., 1998
	- <23 °C	Portugal	Reis, 2003; Teixeira *et al*., 2010; This Study
	- <26 °C	Portugal	Lopes-Lima Pers. data
pH	- 5.9-6.7	Russia	Semenova *et al*., 1992
	- 6.5-7.2	England	Oliver, 2000
	- ≤7.5	Central Europe	Bauer, 1988; WWF, 2005
	- usually neutral to slightly acidic	Central Europe	Geist *et al*., 2006
	- 6.7-8.6	Austria	Moog *et al*., 1998
	- ≤7.6	Portugal	Reis, 2003
	- ≤7.0	Portugal	This Study
Permanganate oxidizability (mg O L^{-1})	- ≤37	Russia	Semenova *et al*., 1992
Conductivity (μS.cm^{-1})	- low overall conductivity (<70)	Finland	Valovirta, 1995
		Central Europe	Bauer, 1986, 1988
		England	Oliver, 2000
	- ≤100	England	Skinner *et al*., 2003
	- < 100-150 μS, 25 °C	Austria / Ireland	Moog *et al*., 1998; Moorkens *et al*., 2000
	- ≤200	Central Europe	Geist *et al*., 2006
	- <50	Portugal	Teixeira *et al*., 2010
	- low overall conductivity (<70)	Portugal	Reis, 2003
	- <40	Portugal	This Study
Suspended solids (mg.L^{-1})	- ≤10 (30 during floods)	Finland	Valovirta, 1998a
	- ≤10	Portugal	Teixeira *et al*., 2010
	- ≤20	Portugal	This Study
	- Oligotrophic conditions	England	Skinner *et al*., 2003

(*Continues*)

Table 1. Continued

A - Ecological requirements		Region	References
Nutrient levels / Trophic state		Austria	Moog *et al.*, 1998
		Central Europe	Bauer, 1988; WWF, 2005; Geist and Auerswald, 2007
		Portugal	Reis, 2003
Nitrate (mg NO_3^-.L^{-1})	- ≤1.0	England	Oliver, 2000; Skinner *et al.*, 2003
	- ≤1.7	Ireland / Austria	Moorkens, 2000
	- 0.5	Central Europe	Bauer, 1986; 1988
	- <2	Portugal	This Study
Calcium (mg $CaCO_3$.L^{-1})	- poor in calcium	Ireland	Moorkens, 1999
		Northern and Central Europe	Boycott, 1936; Geist and Auerswald, 2007
	- 2	Central Europe	Bauer, 1988
	- <10	England	Oliver, 2000
	- poor in calcium	Spain	Rosas *et al.*, 1992
Ammonium (mg NH_4^+ L^{-1})	- <0.10	Ireland	Moorkens, 2000
	- <0.10	Portugal	Teixeira *et al.* 2010; This Study
Phosphate (mg $PO_4^{3-}$$L^{-1}$)	- <0.03	Central Europe / England	Bauer, 1988; Oliver, 2000; Skinner *et al.*, 2003
	- <0.06	Ireland	Moorkens, 2000
	- <0.1	Portugal	Teixeira *et al.*, 2010; This Study
Total phosphorus	- 20-35 mg.m^{-3} (0,02-0,035 ppm)	Austria	Moog *et al.*, 1998
	- < 100 mg.m^{-3}	Portugal	Teixeira *et al.*, 2010
Substrate	- coarse sand and gravel	Russia	Ostrovsky and Popov, 2011
		Ireland	Moorkens, 1996, 1999; Beasley and Roberts, 1999; Wilson *et al.*, 2011
		England	Skinner *et al.*, 2003
		Austria	Moog *et al.*, 1998
	- small patches of stable, clean sand protected between large boulders	Scotland	Young and Williams, 1983; Hastie *et al.*, 2000a; 2003b
	- mixed substrata of boulders, stones and sand	Sweden	Björk, 1962
		England and Germany	Purser, 1985
		Ireland	Chesney *et al.*, 1993
		Northern and Central Europe	Boycott, 1936
	- coarse, well-sorted sediment stabilized by boulders or stones	Northern and Central Europe	Geist and Auerswald, 2007
	- coarse to fine gravel, coarse sand and stones	Spain	Ocharan *et al.*, 1997; Álvarez-Claudio *et al.*, 2000; Outeiro *et al.*, 2008
	- small patches of stable, clean sand protected between large boulders	Portugal	Teixeira *et al.*, 2010
	- clean sand, and granite primary rocks	Portugal	Reis, 2003
Riparian habitat/ Shadow	- margins well-structured/shaded areas	Russia	Ostrovsky and Popov, 2011
		Austria	Moog *et al.*, 1998
	- shade created by overhanging herbaceous vegetation, scrub and bank-side trees	England	Skinner *et al.*, 2003
	- broadleaf/ mixed woodland/ bankside tree cover	Scotland	Hastie *et al.*, 2003b
		Ireland	Baer, 1981; Lucey, 1993; Gittings *et al.*, 1998; Wilson *et al.*, 2011
	- shade of trees	Ireland	Gittings *et al.*, 1998
	- unshaded areas (in very clean waters)	Ireland	Moorkens, 1996
	- shade of trees	Spain	Ocharan *et al.*, 1997; Álvarez-Claudio *et al.*, 2000; Outeiro *et al.*, 2008
		Portugal	Teixeira *et al.*, 2010

(*Continues*)

Table 1. Continued

A - Ecological requirements		**Region**	**References**
Host densities	- High % of *Salmo trutta* 0+ (100 mm) and 1+ (100-150 mm)	Austria	Moog *et al.*, 1998
	- mostly 0+ and 1+ years old	Scotland / Central Europe	Young and Williams, 1984; Bauer and Vogel, 1987; Young, 1991;
	- 0.2 fish m^{-2}	Central Europe	Bauer, 1988
	- Minimum density 0.1 fish m^{-2} (10 fish $100m^{-2}$) for successful glochidia infection	Russia	Ziuganov *et al.*, 1994
	- Minimum density 0.2 fish m^{-2} (20 fish $100m^{-2}$) for successful glochidia infection	Germany / Central Europe	Bauer, 1991; Geist *et al.*, 2006.
	- densities between 5–10 fish $100m^{-2}$ of trout are sufficient for recruitment	Sweden	Österling *et al.*, 2010.
	- 10 fish $100m^{-2}$ have been observed to be sufficient	Europe	Arvidsson *et al.*, 2012
	- usually 0+ and 1+ salmonids	Scotland	Hastie and Young, 2001
	- 3.6-22 (0+ trout.100 m^{-2})	Sweden	Österling, *et al.*, 2008
	- *Salmo trutta* 0.116 ind.m^{-2}	Portugal	This Study
B - Characteristics of viable *M. margaritifera* populations		**Region**	**References**
Age at maturity	- 12-20 years	Russia	Ziuganov *et al.*, 1994
		Austria	Moog *et al.*, 1998
	- 12-13 years	Scotland	Young and Williams, 1984
	- 10-15 years (>65 mm)	England	Skinner *et al.*, 2003
	- 12-15 years (>65 mm)	Scotland	Hastie *et al.*, 2000c
	- 20 years	Germany	Bauer, 1987
	- >6 years (55–60 mm)	Spain	San Miguel *et al.*, 2004
Growth rates (k, year^{-1})	- 0.023-0.063	NW Russia	Ziuganov *et al.*, 1994
	- 0.023-0.075	Scotland	Hastie *et al.*, 2000b
	- 0.016-0.107	NW Ireland	Beasley, 1996
	- 0.054-0.111	W Ireland	Ross, 1984
	- 0.054-0.124	Bavaria	Bauer, 1991, 1992
	- 0.018-0.108	Overall	Bauer, 1992
	- >0.1 (on average)	Spain	San Miguel *et al.*, 2004
Density for *M. margaritifera* habitat	- >100 mussels m^{-2} and 500 individuals per 500 m stretch of river	Finland	Valovirta, 1990
	- 0.27-4 mussels m^{-2}	Sweden	Österling *et al.*, 2008
	- 2.5–14.5 mussels m^{-2} (median densities)	Scotland	Hastie *et al.*, 2000a
	- optimal density ≥1 mussels m^{-2}	Scotland	Hastie *et al.*, 2003a,2003b, 2004
		Finland	Valovirta, 1998b
		Scotland	Cosgrove *et al.*, 2000
	- 10 mussels m^{-2} within the quadrats in the 50 m transect	United Kingdom	Young *et al.*, 2003
	- 14,5-76 ind m^{-2}	Spain	Álvarez-Claudio *et al.*, 2000
	- 0.057 mussels m^{-2}	Spain	Velasco *et al.*, 2002
	- 0.11 mussels m^{-2}	Spain	Morales *et al.*, 2004
	- 0.09–50 mussels m^{-2}	Portugal	Reis, 2003
Recruitment (proportion of juvenils) %	- minimum density of 500 adults per 100 m of river is the critical level for fertility needed for successful recruitment to take place	Finland	Valovirta, 1990
	- 20 % less than 20 years	Scotland	Hastie *et al.*, 2000c
	- 20 % less than 20 years (<65 mm long) and at least some of below 10 years old (≈30 mm long)	Scotland	Young *et al.*, 2001a, b
	- presence of individuals <65 mm long are 'functional"	Scotland	Cosgrove *et al.*, 2000
	- ≥25% less than 20 years old	Scotland	Hastie and Cosgrove, 2002
	- >2 juvenile mussels (≤5 years old) per m^2	Northern and Central Europe	Geist and Auerswald, 2007
	- 50% of the population in the River Narcea is less than 20 years old	Spain	Álvarez-Claudio *et al.*, 2000
	- 30 % less than 20 years old	Southern Europe	Bauer, 1988

