Estuaries and Coasts (2008) 31:1198–1207
DOI 10.1007/s12237-008-9090-3

Factors Affecting *Pisidium amnicum* (Müller, 1774; Bivalvia: Sphaeriidae) Distribution in the River Minho Estuary: Consequences for its Conservation

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Received: 22 April 2008 / Revised: 29 July 2008 / Accepted: 18 August 2008 / Published online: 12 September 2008

Abstract The bivalve *Pisidium amnicum* (Müller 1774) is a common species in several European freshwater ecosystems. However, few Iberian watersheds are colonized by this species, and the River Minho estuary is possibly the Iberian aquatic ecosystem with the larger population. In October 2004–2007, investigations on spatial and temporal variations in *P. amnicum* abundance and biomass were carried out at 16 sites along the River Minho tidal freshwater wetlands. Mean abundance and biomass per site ranged from 0 to 750 ind m^{-2} and 0 to 7.42 g AFDW m^{-2}, respectively. A clear decrease in the spatial distribution, abundance, and biomass was observed during the 4-year assessment. Furthermore, a stepwise multiple regression model showed that organic matter and conductivity explained 50.2% of the variation in *P. amnicum* abundance (R^2=0.502, $F_{[2,\ 15]}$=7.569, p=0.005). Ecological knowledge is essential to the implementation of future conservation plans for *P. amnicum*, and the results of this study are of paramount importance to identify habitats that should be protected in order to preserve this species and provide scientific reference that may be useful in the development of management and/or restoration plans.

Keywords River Minho · *Pisidium amnicum* · Abundance · Biomass · Abiotic factors · Conservation

Introduction

Threats to animal freshwater species (e.g., habitat loss, climate change, pollution, introduction of nonindigenous invasive species (NIS), presence of impoundments, flow regularizations, overexploitation) are accelerating and are important enough to change ecosystems functioning and drive species to extinction (Dudgeon et al. 2006). Indeed, the loss of biodiversity in freshwater ecosystems constitutes a relevant topic in international conservation studies, and several works conducted in the last years claimed an urgent increase of interest on this subject (Dudgeon 2000; Strayer et al. 2004). Although biodiversity seems to be decreasing in freshwater ecosystems, there is still a general unawareness about this issue, which is much more evident when we are dealing with invertebrates that have a disproportional minor attention than vertebrates (Strayer 2006). This apparent lack of concern might be disastrous because invertebrate species performed essential functions in aquatic ecosystems (e.g., food resource to higher trophic levels, involvement in facilitation and

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ecosystem engineering processes, and regulation of primary production, decomposition, water clarity, and nutrient cycling).

Within the freshwater benthos, mollusks are fundamental (and threatened) taxa in terms of biodiversity and ecosystem functioning (Gutiérrez et al. 2003; Spooner and Vaughn 2006; Vaughn and Spooner 2006). Despite their importance, the number of studies carried out with these animals continues to be scarce, and numerous ecological gaps still exist. Nevertheless, since the 1990s, a growing interest on these animals seems to be reappearing, mainly focusing on freshwater mussels. These remarkable organisms are now the core of conservation and ecological studies, particularly in North America (Lydeard et al. 2004; Strayer et al. 2004). Other freshwater bivalves such as species from the Sphaeriidae family also deserve special attention. However, the number of studies about these small bivalves is insufficient, and as a result, the availability of information is still limited (Watson and Ormerod 2005).

The Sphaeriidae *Pisidium amnicum* is a common freshwater European species, occurring mainly in the northern countries (Holopainen 1979; Dyduch-Falniowska 1983; Zettler 1996; Zettler and Daunys 2007). This bivalve is described as having the European southern limit in the Iberian Peninsula, where the River Minho corresponds to its southernmost distribution limit. In fact, the larger Iberian population was found in the River Minho tidal freshwater wetlands (TFWs) (Araujo et al. 1999). *P. amnicum* is hermaphroditic, incubating their eggs in brood sacs inside the inner gills, and each bivalve releases several juveniles, with nearly 2 mm, directly to the sediment (Dillon 2000). The number of incubated larvae has a latitudinal difference. The southern populations have a maximum of 73 (Portugal and Spain, River Minho; Araujo et al. 1999), and the northern population a maximum of 12 (Finland, lake Pääjärvi; Holopainen 1979). Their size and life span also have latitudinal differences (Holopainen 1979; Araujo et al. 1999).

One of the conservational problems identified by previous studies performed in the River Minho is the loss of indigenous molluskan diversity, particularly after the introduction of the nonindigenous bivalve *Corbicula fluminea* (Müller 1774) (Sousa et al. 2005, 2007b, 2008a, in press). The main goal of this study was to investigate the evolution of the abundance and biomass of *P. amnicum* between 2004 and 2007. In addition, these data were used to develop a model describing the relationship between abiotic factors and abundance of *C. fluminea* with abundance of *P. amnicum* in the River Minho TFWs. This information will be valid to identify environmental stressors and favorable abiotic conditions that control the presence of *P. amnicum* and to propose conservation measures for future reestablishment.

Materials and Methods

Study Area

The River Minho estuary has a maximal length of 40 km with a tidal freshwater portion of nearly 30 km. This mesotidal estuary is partially mixed; however, during periods of high floods, it tends to advance towards a salt wedge estuary (Sousa et al. 2005). Its main environmental and ecological characteristics were object of recent studies (Sousa et al. 2005, 2007b, 2008a, in press). Special attention has been paid to impacts on this estuarine ecosystem (e.g., habitat loss, introduction of NIS, climate change, flow regularization, fisheries, pollution) and on estuarine macrozoobenthic assemblages' distribution and its relation with the abiotic factors.

Sampling and Laboratory Analysis

Samples were collected at 16 sites along the River Minho TFWs (Fig. 1) at high tides in October 2004–2007 (after *P. amnicum* recruitment season). Five replicates for each site (one for sediment analysis and four for biological analysis) were collected with a 0.05 m^2 van Veen benthic grab (maximum volume of 0.005 m^3) except for the 2004 samples, when three replicates were collected per site (one for sediment analysis and two for biological analysis).

At each site, temperature (T), conductivity (CND), total dissolved solids (TDS), redox potential (ORP), salinity (S), dissolved oxygen (DO), and pH were registered close to the bottom with the multiparameter probe YSI 6820.

Water samples were collected at intermediate depths to determine concentration of nitrites, nitrates, ammonia, phosphates, and water hardness. They were kept at cool temperatures in the dark until subsequent laboratorial processing. The concentrations relative to each of these parameters were determined by colorimetric methods using the Palintest 270 standard photometer 7000.

Sediment granulometry, which was divided in six size classes [very coarse sand (VCS), coarse sand (CS), medium sand (MS), fine sand (FS), very fine sand (VFS) and silt + clay (S + C)] and the organic matter (OM) content of sediment were determined using the methodology described in Sousa et al. (2007a). All values are expressed as percentage of each sample weight.

Biological samples obtained from the 16 sites were sieved through a mesh of 500 µm, and *P. amnicum* specimens were sorted. The abundance was determined and the shell length of each specimen was measured with a digital caliper (± 0.01 mm). *P. amnicum* biomass was calculated using the ash free dry weight method (AFDW) as in the methodology described in Sousa et al. (2006). Abundance and biomass (AFDW) of the nonindigenous

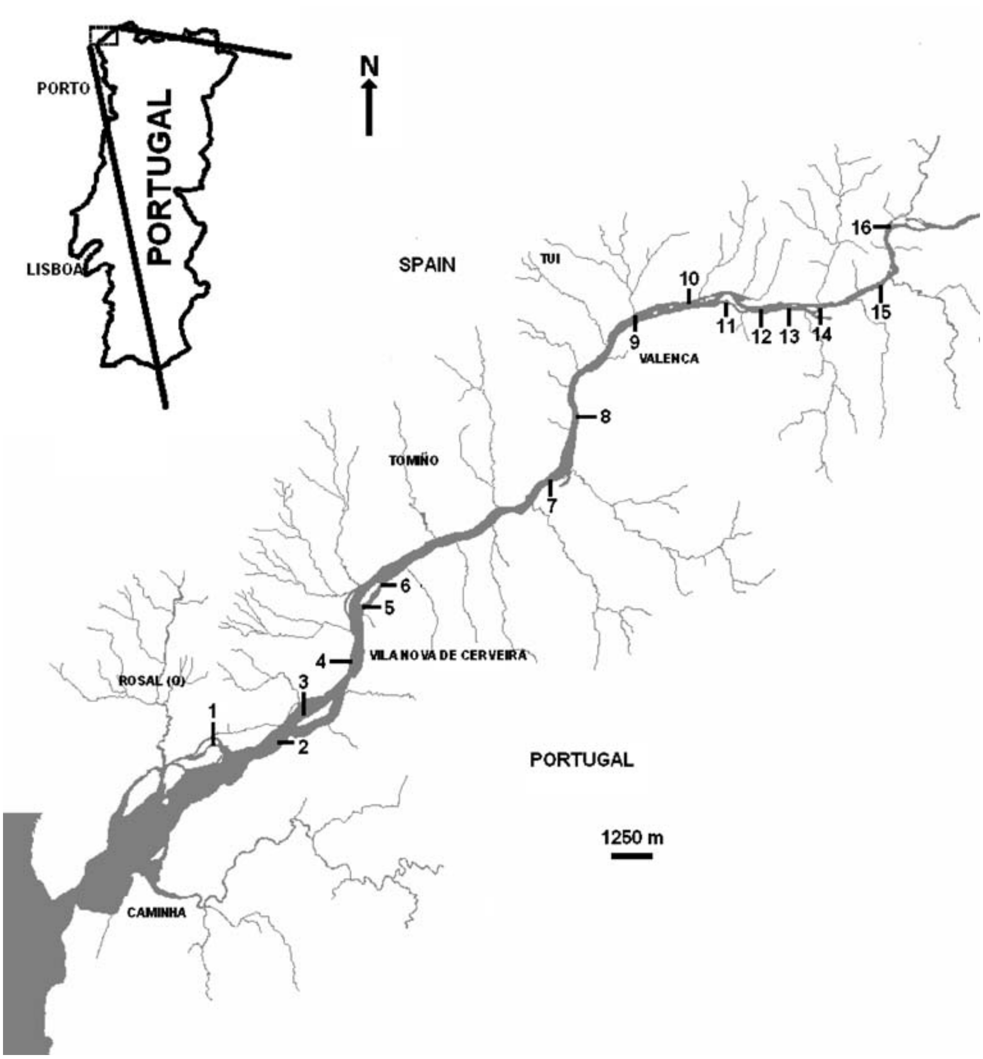

Fig. 1 Map of River Minho estuary showing the 16 sites location

bivalve *C. fluminea* were also determined, and detailed information about the abundance, biomass, and distribution of this species is in Sousa et al. (2008a, b).

Statistical Analysis

A cluster analysis was performed using the monthly River Minho inflow measured at Foz do Mouro hydrometric station (01G/02H; Water Institute of Portugal—INAG) to determine the degree of annual river inflow (data from 1990 onwards). The Ward's method was the chosen amalgamation rule, and the joining clustering was applied using the dissimilarities or distances between variables to form the clusters. The Euclidean distance was used because the distances between any two objects are not affected by outliers (StatSoft 2004).

Significance tests for variations in the abiotic factors measured between sites and years were undertaken using a two-way crossed ANOSIM2. Additionally, environmental characterization of the area was performed using principal components analysis (PCA), applied to the mean values measured during the 4 years. These analyses were performed with PRIMER 5.0 (Clarke and Warwick 2001).

A two-way analysis of variance (ANOVA) followed by a posteriori Tukey HSD test was performed to check differences in *P. amnicum* abundance between sites and years, using SigmaStat 2.03. Only the three sites with higher abundance (sites 11, 12, and 15) were used to assess significant spatial and temporal changes. Raw data was used in the two-way ANOVA analysis. In order to guarantee normal distribution and variance homogeneity data were $\sqrt{abun+1}$ transformed. A confidence interval of 95% was set, and the power of the analysis was assessed with α=0.05.

A stepwise multiple regression was computed to estimate the coefficients of linear equation, involving the independent variables that best predict the value of *P. amnicum* abundance, using the software SPSS 15. To avoid violating assumptions underlying regression analysis and to directly test for the effect of environmental variables on *P.*

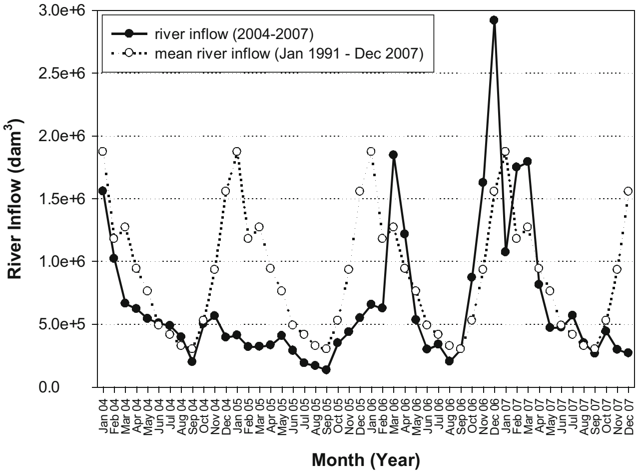

Fig. 2 Monthly variation of the river inflow measured at Foz do Mouro hydrometric station (data from INAG) between January 2004 and December 2007. The annual river inflow based on mean data collected between January 1991 and December 2007 was also given

amnicum abundance variation, the sites with zero *P. amnicum* were removed (Jones and Ricciardi 2005). For data analysis, bivalve abundance was $\log_{10}$ transformed to normalize and stabilize variances. Accordingly, each predictor variable was also observed in detail to determine if a transformation improved their distribution. Thus, temperature (log.T), conductivity (log.CND), total dissolved solids (log.TDS), redox potential (log.ORP), salinity (log.S), dissolved oxygen (log.DO), pH (log.pH), nitrites (log.nitrites), nitrates (log.nitrates), ammonia (log.ammonia), phosphates (log.phosphates), and hardness (log.hardness) were $\log_{10}$ transformed. Variables in percentage (i.e., sediment granulometry: asin.VCS, asin.CS, asin.MS, asin.FS, asin.VFS, asin.S + C; and organic matter: asin.OM) were arcsine transformed, as recommended by Zar (1999). In addition to the abiotic factors, abundance of *C. fluminea* [data from Sousa et al. (2008a, b)] was added to the analysis, as well as the type of hydrological year, i.e., very low, low, mean, high, and very high river inflow, to which a numerical score was attributed varying between 1 and 5, respectively.

Results

Abiotic Characterization

From January 2004 to December 2007, the river inflow varied between 133,600 dam^3 (September 2005) and 2,918,399 dam^3 (December 2006) (Fig. 2). The cluster analysis identified 2005 as a year of very low river inflow (score 1), 2004 and 2007 as years of low inflow (score 2), and 2006 as a year of mean river inflow year (score 3) (Fig. 3).

Detailed characterization of the abiotic factors measured at each site along the 4 years is in Sousa et al. (2008b, Table 1). The ANOSIM2 tests based on abiotic factor similarities showed significant differences between sites (R=0.582; p<0.001), but not between years (R=0.065; p=0.103). Since ANOSIM2 test did not detected differences between years, mean values were used in the PCA (Fig. 4). Three main areas were distinguished along the estuarine gradient: Group 1—comprising sites 1 to 5; Group 2—comprising sites 6 to 12; and Group 3—comprising sites 13 to 16. These groups appeared distrib-

Fig. 3 Cluster diagram applied to the annual river inflow measured at Foz do Mouro hydrometric station

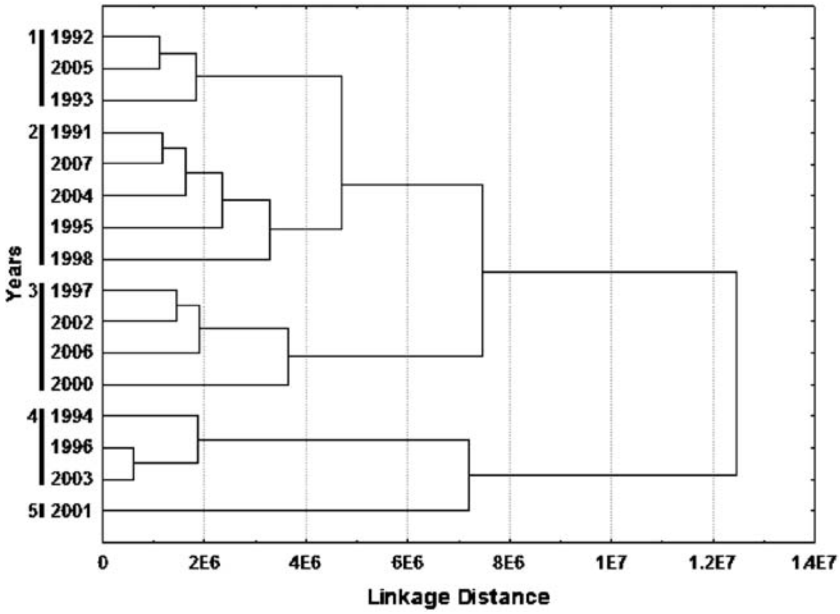

Table 1 Results of two-way ANOVA tests for differences in *P. amnicum* abundance between sites and years

Source of variation	*dof*	SS	MS	*F*	*p* Value
Year	3	885.789	295.263	14.827	<0.001
Site	2	659.853	329.927	16.568	<0.001
Year × site	6	653.032	108.839	5.466	<0.001
Residual	30	597.399	19.913		
Total	41	2558.008	62.390		

uted along an abiotic gradient, from the lower to the upper estuarine areas. Group 1 was characterized by high values of conductivity, salinity, and total dissolved solids. In this estuarine area, sandier sites with low organic matter content were predominant except for the site 2, which presented fine sediments and high level of organic matter. Group 2 was characterized by fine deposits with high organic matter content. In addition, this group showed peak concentrations of nitrates, nitrites, ammonia, and phosphates at site 9. Finally, Group 3 was characterized by sandier deposits with low organic matter content, low nutrient concentrations, and low tidal influence.

Biotic Characterization

P. amnicum was only collected in areas upstream site 5. Mean abundance ranged between 0 and 750 ind m^{-2} (site 12, 2004) (Fig. 5A) and mean biomass between 0 and 7.42 g AFDW m^{-2} (site 12, 2004) (Fig. 5B).

There were significant differences in the mean value of *P. amnicum* abundance between years ($p<0.001$), sites ($p<0.001$), and "Year vs Site" ($p<0.001$) at the three sites where the abundance was higher: sites 11, 12, and 15 (Table 1). There were significant differences between 2004 and 2006 ($p<0.001$) and 2007 ($p<0.001$) and between 2005 and 2006 ($p=0.050$) and 2007 ($p<0.001$) (Table 2). There were also significant differences between sites 15 and 11 ($p<0.001$) and 12 ($p<0.001$) (Table 3). Within each site, it was possible to identify significant differences between 2004 and 2006 ($p=0.014$) and 2007 ($p=0.002$) at site 11. At site 12, significant differences were detected between all years ($p<0.036$), except between 2006 and 2007 ($p=0.475$). At site 15, no significant differences were detected between years ($p>0.653$) (Table 4).

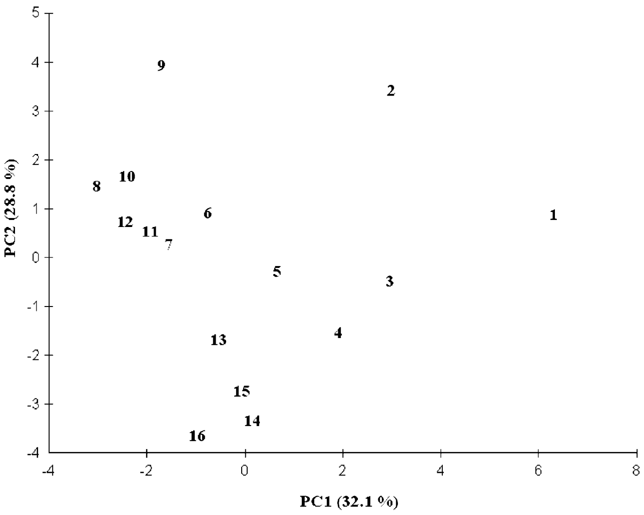

Fig. 4 PCA showing the plotting of the 16 sites. The percentage of variability explained by the principal axes is given

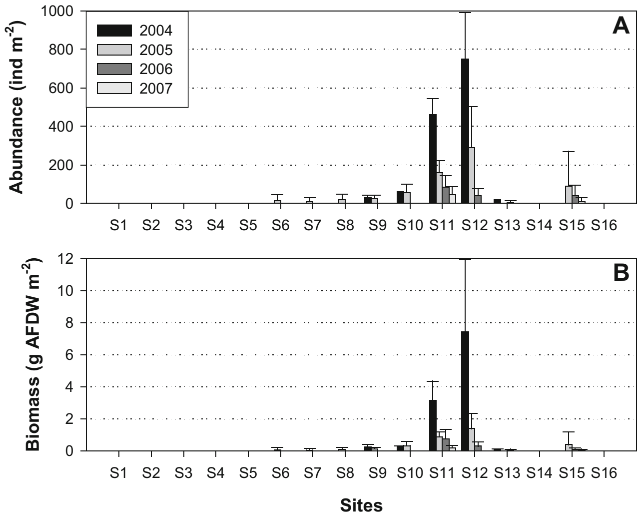

Fig. 5 Annual and spatial variation of *P. amnicum* mean abundance (ind m^{-2}) (**A**) and mean biomass (g AFDW m^{-2}) (**B**). The confidence bands represent the standard deviation

The multiple stepwise regression model showed that organic matter (expressed as asin.OM) and conductivity (expressed as log.CND) were the independent variables that best explained the variation of *P. amnicum* abundance (expressed as logAbundance; $R^2=0.502$; $F_{2,15}=7.569$; $p=0.005$) (Table 5). The asin.OM explained 29.9% of the variation of *P. amnicum* $\log_{10}$ abundance and showed a positive coefficient; thus, its abundance was greater for higher values of OM (Fig. 6A). The log.CND explains 19.1% of *P. amnicum* $\log_{10}$ abundance variation (Fig. 6B), and a negative relationship was established.

Table 2 Results of Tukey tests for differences in *P. amnicum* abundance between years

Comparision	Diff of means	p	Q	*p* Value
2004 vs 2005	5.162	4	3.272	0.118
2004 vs 2006	9.993	4	6.334	<0.001
2004 vs 2007	13.468	4	8.573	<0.001
2005 vs 2006	4.831	4	3.750	0.050
2005 vs 2007	8.306	4	6.448	<0.001
2006 vs 2007	3.475	4	2.698	0.246

Table 3 Results of Tukey tests for differences in *P. amnicum* abundance between sites

Comparison	Diff of means	p	Q	*p* Value
Site 11 vs site 12	0.343	3	0.275	0.979
Site 11 vs site 15	8.617	3	6.909	<0.001
Site 12 vs site 15	8.960	3	7.184	<0.001

Discussion

Abiotic Characterization

In the last 17 years, 3 years (1992, 1993, and 2005) recorded very low river inflow. In 2005, a severe drought occurred in Portugal, causing serious deleterious impacts on many estuarine communities including planktonic (Marques et al. 2007), benthic (Cardoso et al. 2008), and ichthyic (Dolbeth et al. 2007; Martinho et al. 2007). This drought probably affected the abundance of *P. amnicum* in the River Minho TFWs (see below).

The PCA analysis revealed a clear spatial pattern along the estuarine area, discriminating three main groups, which are in accordance with earlier studies (Sousa et al. 2008a, in press). This spatial pattern is related with different sediment characteristics and with a clear estuarine gradient, mainly related with salinity, conductivity, and total dissolved solids. Nutrient concentrations also support the discrimination of the Group 2 (presenting higher values) from the others. In detail, Group 1 has greater tidal influence and sandier deposits; Group 2 is probably associated with increased organic pollution due to the influence of a River Minho tributary (River Louro) and is richer in finer sediments and organic matter content; finally, Group 3 is less polluted and have residual tidal influence.

Table 4 Results of Tukey tests for differences in *P. amnicum* abundance between years in sites 11, 12, and 15

Comparision	Diff of means	p	Q	*p* Value
Site 11				
2004 vs 2005	8.918	4	3.264	0.119
2004 vs 2006	12.642	4	4.626	0.014
2004 vs 2007	15.530	4	5.683	0.002
2005 vs 2006	3.724	4	1.669	0.644
2005 vs 2007	6.611	4	2.963	0.178
2006 vs 2007	2.887	4	1.294	0.797
Site 12				
2004 vs 2005	11.068	4	4.050	0.036
2004 vs 2006	21.628	4	7.915	<0.001
2004 vs 2007	26.226	4	9.597	<0.001
2005 vs 2006	10.559	4	4.733	0.011
2005 vs 2007	15.158	4	6.793	<0.001
2006 vs 2007	4.598	4	2.061	0.475
Site 15				
2004 vs 2005	4.500	4	1.647	0.653
2004 vs 2006	4.291	4	1.570	0.686
2004 vs 2007	1.351	4	0.494	0.985
2005 vs 2006	0.209	4	0.094	1
2005 vs 2007	3.149	4	1.411	0.752
2006 vs 2007	2.941	4	1.318	0.788

Biotic Characterization and Ecological and Conservation Significance

P. amnicum preferentially colonizes rivers that do not present considerable seasonal hydrological oscillations, being a common species in lowland areas and limnetic portions of estuarine ecosystems (Killeen et al. 2004). In accordance with this, the results of the present study showed higher values of abundance and biomass in the upstream areas of the River Minho estuary. The results of the stepwise multiple regression model combining abiotic data, abundance of *C. fluminea*, and abundance of *P. amnicum* indicated that sites with high organic matter and low conductivity supported higher abundance of *P. amnicum*. Organic matter is likely to be an important factor influencing the distribution of *P. amnicum* because this clam utilizes pedal feeding to extract organic matter from the interstitial water and sediment, which can be rich in macroalgae detritus, submerged vegetation detritus, and bacteria (Holopainen 1979; Hakenkamp and Palmer 1999; Hakenkamp et al. 2001; Vaughn and Hakenkamp 2001). Hence, sites with high organic matter content can sustain high abundance and biomass of *P. amnicum*. Furthermore, one should observe the correlation existing between organic matter and fine sediments (data not shown). So, sediment characteristics could be also important in the distribution of this benthic species, a fact already established for other freshwater bivalves (Mellina and Rasmussen 1994; Jones and Ricciardi 2005; Sousa et al. 2008a). In this study, conductivity was negatively correlated with abundance of *P. amnicum*, and this species seems to prefer the upper estuarine areas where conductivity is lower because of a decreased tidal influence. In fact, high conductivity can be responsible for gross osmoregulatory problems and changes in the ionic ratios of freshwater molluscs (Dillon 2000).

Although organic matter and conductivity appear to be important to the distribution of *P. amnicum* in the River Minho TFWs, it could also be influenced by other abiotic factors not assessed in this study. For instance, current velocity could exert potential influence in juveniles' distribution and migration rates. Nevertheless, sediment granulometry reflect the general hydrological conditions, including current velocity. In addition to this, biotic factors such as predation, competition, parasitism, and presence of

Table 5 Stepwise multiple regression models developed to predict (log) *P. amnicum* abundance across sites from two independent predictor variables, organic matter (asin.OM) and conductivity (log.CND) (R^2=0.502, $F_{[2,\ 15]}$=7.569, p=0.005)

	B	St. error	Beta	T	*p* Value	R^2	SS	df	MS	F	*p* Value
Model											
Constant	5.986	2.111		2.836	0.013	0.502					
asin OM	5.987	1.920	0.568	3.117	0.007						
log CND	−2.422	1.072	−0.412	−2.260	0.039						
Source of variation											
Regression							2.625	2	1.312	7.569	0.005
Residual							2.601	15	0.173		
Total							5.225	17			

The ANOVA results are also shown.

submerged vegetation may also affect the distribution of *P. amnicum*. Future studies should examine these abiotic and biotic factors and possibly add them to the stepwise multiple regression model. On the other hand, since this study was confined to one Iberian aquatic ecosystem and some results may be restricted solely to the River Minho TFWs, caution should be taken when applying this model to other areas, as this population possibly corresponds to the southernmost distribution limit, and these TFWs are almost completely dominated by *C. fluminea*.

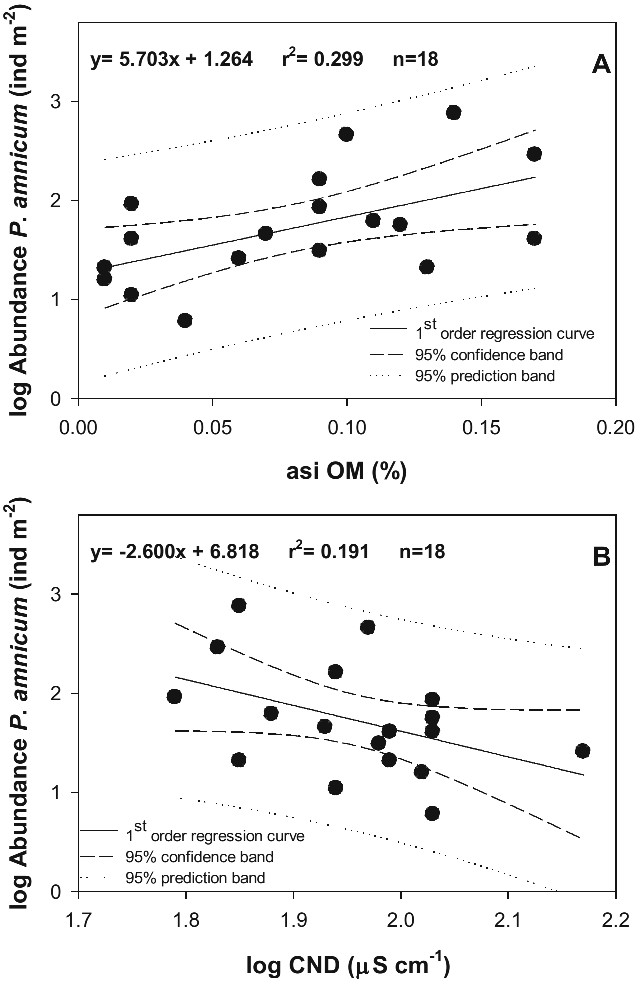

Fig. 6 Relationship between *P. amnicum* abundance (log transformed) and organic matter content (asin transformed) (**A**) and conductivity (log transformed) (**B**)

During the execution of this study, *P. amnicum* could not be found in the lower areas of the River Minho TFWs (sites 1 to 5). At sites 6–8, its abundance was very low. However, previous studies report the occurrence of this mollusk in all TFWs until the 1990s (Nobre 1941; Araujo et al. 1993, 1999). Thus, *P. amnicum* appears to have suffered a dramatic reduction of its spatial distribution since this time. The reasons behind this fact are yet to be investigated. In addition, these earlier studies only registered the presence or absence of *P. amnicum* and the abiotic characterization was very poor, which makes the evaluation of the ecological and environmental changes rather speculative. Pollution and loss of habitat do not seem to represent important pressures in this estuarine area. In contrast, this estuary has been suffering the impacts caused by the introduction of several NIS, particularly *C. fluminea* (Araujo et al. 1993; Sousa et al. 2005, 2007b, 2008a, b, in press). Although this invasive clam could be affecting the abundance, biomass, and spatial distribution of *P. amnicum*, up to now, no study investigated the existence of competition between these two bivalves or other kind of interspecific relationship that could result in the decline of the indigenous species. However, a previous study showed a clear decrease in the abundance and distribution of *P. amnicum*, mainly observed in the lower areas of the River Minho TFWs, concomitant with the introduction and rapid expansion of *C. fluminea* after 1989 (Araujo et al. 1993). Another alternative or cumulative hypothesis is a possible increase of conductivity due to decrease in the river discharge. This situation is probably an outcome of dams' construction and climate change and might extend the influence of seawater to upstream areas. Since the occurrence of this clam is negatively correlated with conductivity, the possible increase in the levels of this abiotic factor might have intensified the environmental stress on this population.

A rapid reduction in the abundance and biomass of *P. amnicum* was noted during the last 4 years. The pressures imposed by the strong drought of 2005 probably were responsible for this decline. The alterations in the abiotic features caused by this drought included: low flow, high water temperatures, low level of dissolved oxygen, and decrease in the redox potential (Sousa et al. 2007b, 2008a, b, in press). These environmental changes were responsible for substantial mortality of benthic species, including *P. amnicum* (Sousa et al. 2007b). The synergistic effects of these abiotic alterations together with the fast recovery of the NIS *C. fluminea* probably had detrimental effects on the local *P. amnicum* population. These two bivalves have different life cycle strategies, and *C. fluminea* could have taken advantage over *P. amnicum* when these changes occurred, as this invasive clam presents high reproductive rates, a typical characteristic of species with r-strategy life cycle (McMahon 2002). Indeed, a unique Asian clam can release almost 70,000 live offspring per year, while *P. amnicum* releases no more than a few dozens (Araujo et al. 1999; Keller et al. 2007). Furthermore, after the impact, the system was rapidly occupied by *C. fluminea*, possibly interfering with the recruitment success of *P. amnicum*. The current data show a significant decline of *P. amnicum* abundance during the last 4 years. Since there were no signs of recovery from this dwindling trend, the extirpation of *P. amnicum* from the River Minho basin is a scenario to be taken into consideration.

In the last years, special attention has been paid to the decrease of the biodiversity and the consequences of that on the ecosystem functioning (Daily et al. 2000; Loreau et al. 2002; Covich et al. 2004). The theory states that the ecosystem functioning is less affected by the loss of species in systems with great biodiversity than in systems with low biodiversity (Tilman 1999; Yachi and Loreau 1999). Considering the River Minho TFWs does not shelter a great biodiversity, the disappearance of *P. amnicum* from this system could cause the loss of important functions and services provided by this organism (e.g., food resource for higher trophic levels; bioturbation of the top layer of the sediments; nutrient cycling through excretion, biodeposition of feces, and pseudofeces; shells can be important for ecosystem engineering processes; Holopainen 1979; Vaughn and Hakenkamp 2001). The multiple regression model identifies the upstream areas of River Minho estuary, with high organic matter content and low conductivity, as habitats that should be preserved. These habitats are of vital importance not only for *P. amnicum* but also for several other mollusks that occur in the same patches, which include freshwater mussels (*Psilunio litorallis*, *Anodonta anatina*, *Unio pictorum*) and gastropods (*Lymnaea peregra*, *Bithynia tentaculata*, *Valvata piscinalis*, *Ancylus fluviatilis*; Sousa et al. 2007b, 2008b, in press).

Conclusion

Until around 20 years ago, *P. amnicum* colonized all the River Minho TFWs. Nowadays, this species has almost totally disappeared from the lower estuarine areas, subsisting in small patches located mainly in the upper limit of the tidal influence, in very low abundance and biomass. The reasons that may have caused this declining trend are not clear. Nevertheless, the introduction of *C. fluminea* may be posing a serious threat to this indigenous bivalve, probably by direct competition (i.e., *P. amnicum* is restricted to sites having high organic matter content where the probable competition between the two species for food resources is lower) or by altering the ecological characteristics of this ecosystem. In addition, the synergistic effects produced by climate change and decrease in the river discharge may have also contributed to this decline.

The results of this study enhanced the knowledge on *P. amnicum* ecology and provided scientific reference that may be important for future conservation plans and of paramount importance to define habitats that should be protected. This information is essential in the development of management and/or restoration plans to be implemented in the River Minho TFWs.

Acknowledgements This work was funded by a Ph.D. grant to Ronaldo Sousa (SFRH/BD/18426/2004) and a Postdoctoral grant to Pedro Morais (SFRH/BPD/40832/2007) from the Portuguese Foundation for Science and Technology. Special thanks are addressed to Aline da S. Cerqueira-Holt and Sérgia Dias for helpful suggestions and discussions and Eduardo Martins, Gonçalves, and Hugo Santos for technical assistance.

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