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Rapid decline of the greater European peaclam at the periphery of its distribution

Ronaldo Sousa[1,2]*, Martina Ilarri[2,3], Allan T. Souza[2,3], Carlos Antunes[2,4] and Lúcia Guilhermino[2,3]

[1] CBMA – Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[2] CIMAR-LA/CIIMAR – Centre of Marine and Environmental Research, Rua dos Bragas 289, 4050-123 Porto, Portugal
[3] ICBAS – Institute of Biomedical Sciences of Abel Salazar, University of Porto, Department of Populations Studies, Laboratory of Ecotoxicology, Lg. Prof. Abel Salazar, 2, 4099-003 Porto, Portugal
[4] Aquamuseum of the Minho River, Parque do Castelinho, 4920-290 Vila Nova de Cerveira, Portugal

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Abstract – Extirpation or even extinction of freshwater invertebrate species is a neglected conservation issue; declines in abundance and spatial distribution for freshwater invertebrates are far less documented than for vertebrate species. In the Minho River tidal freshwater wetlands (northwest of Iberian Peninsula), a rapid decline in density and biomass of the bivalve *Pisidium amnicum* was recorded at 16 different sites over seven years, from 2004 to 2010, without any sign of a potential recovery. Mean density values reached more than 80 ind.m^{-2} in 2004, but declined to less than 1 ind.m^{-2} in 2009 and 2010. An identical declining trend was observed for biomass. A significant reduction in the spatial distribution also occurred. The abiotic changes resulting from the 2005 heat wave and possibly the negative interactions imposed by the non-indigenous invasive bivalve *Corbicula fluminea* were the main factors responsible for the declining trends. Given the very low density, *P. amnicum* is facing a serious risk of extirpation in this ecosystem and conservational measures are urgently needed.

Key words: Conservation / extirpation / heat wave / Minho River / *Pisidium amnicum*

Introduction

Freshwater ecosystems are among the most threatened areas on the planet in terms of biodiversity loss (Strayer and Dudgeon, 2010; Vörösmarty *et al*., 2010). Despite this growing evidence, there is still disproportionately little research on these ecosystems, relative to terrestrial systems (France and Rigg, 1998; Sala *et al*., 2000; Lawler *et al*., 2006). Although freshwater ecosystems foster for important ecological functions and services (*e.g.* flood protection, water quality improvement, carbon storage, nutrient cycling, species habitats, food sources, navigation routes, recreational activities and hydrologic connectivity enhancement; Malmqvist and Rundle, 2002), these areas are facing threats resulting in habitat degradation and biodiversity loss (Higgins *et al*., 2005; Zedler and Kercher, 2005). Most of these threats are due to human activities that include extensive habitat deterioration caused by sediment loading and organic pollution, toxic contamination from municipal and industrial sources, stream fragmentation and channelization, flow regulation by dams, dredging activities, overexploitation of resources, introduction of non-indigenous invasive species, climate change and exploitation of water for consumption, irrigation and electricity generation (Malmqvist and Rundle, 2002). These anthropogenic activities have resulted in high extinction rates that are likely underestimated since some extinctions, both at regional and global scales, are not reported due to lack of knowledge and/or taxonomic expertise, especially for less studied taxa (Ricciardi and Rasmussen, 1999; Dudgeon *et al*., 2006).

Freshwater bivalves are fundamental in terms of biodiversity and ecosystem functioning (*e.g.* food resource to higher trophic levels, some species are parasites of fishes, clearing the water and creating habitat for other benthic species; Spooner and Vaughn, 2006; Vaughn and Spooner, 2006) and are also recognized as a faunal group at a high risk of extinction (Lydeard *et al*., 2004; Strayer *et al*., 2004). However, the number of studies carried out with these animals continues to be scarce and numerous ecological and conservation gaps still exist (Watson and Ormerod, 2005; Régnier *et al*., 2009).

*Corresponding author: ronaldo.sousa@ciimar.up.pt

The sphaeriid *Pisidium amnicum* is a common freshwater species in Europe, occurring mainly in the northern and central European countries (Holopainen, 1979; Dyduch-Falniowska, 1983; Zettler, 1996; Zettler and Daunys, 2007). *P. amnicum* is the largest species in the genus, reaching 11 mm, and is hermaphroditic with facultative autogamy and ovoviviparity incubating their eggs in brood sacs inside the inner gills. Each individual can release several juveniles, up to 2 mm long, directly to the sediment (Dillon, 2000). Both iteroparous and semelparous populations exist, and the number of incubated larvae may vary, since the southern European population (Portugal and Spain, Minho River) has a maximum of 73 (Araujo *et al*., 1999) and the northern population (Finland, Lake Pääjärvi) has a maximum of 12 (Holopainen, 1979). Their size and life span (1–3 years) also have latitudinal differences (Holopainen, 1979; Araujo *et al*., 1999) and in the Minho River the life span was estimated to be about two years (Sousa *et al*., 2008b). Growth was continuous throughout the life cycle with a clear acceleration in the first months, which were coincident with the spring and summer periods (Sousa *et al*., 2008b). *P. amnicum* lives at the surface of the sediments and obtains nutritional resources by filtering food from the sediment and interstitial water (Holopainen, 1979).

This study focuses on changes at the population level induced or potentiated by extreme climate events, using a population of the freshwater bivalve *P. amnicum* in the Minho River tidal freshwater wetlands (TFWs) (northwest of the Iberian Peninsula) as a case study. This population, at the southern limit of the species distribution in Europe, experienced a rapid decline after 2005. The main factors that seem to have contributed to this rapid decline are discussed, with the hope that this study can help to increase the attention given to the dramatic decline that several freshwater invertebrates have been facing in the last few decades.

Material and methods

Study area

The Minho River estuary is located in the northwest of the Iberian Peninsula and has a maximum length of approximately 40 km during summer/early autumn. This estuarine area is part of a Natura 2000 site that includes all the international section of the river. This study was conducted in the Minho River TFWs with a length of approximately 30 km (Fig. 1). The studied area is very shallow (few sites have more than 5 m depth) and includes biotypes with mobile and rocky substrata. River walls and other landscape features provide rocky substrates. Biotypes with mobile substrata occur in intertidal and subtidal areas and comprise areas close to the banks, extensive sand and mud flats, inlets that form small bays and as marsh existing in the margins and on the various islands. This estuarine area is described in more detail in earlier studies including the characterization of the benthic and epibenthic assemblages along the estuarine gradient (Sousa *et al*., 2005, 2007, 2008c, 2008e; Costa-Dias *et al*., 2010).

Sampling strategy and laboratory analysis

There were two different sampling strategies: (i) a monthly sampling programme from January 2005 to August 2006 conducted at three different sites (sites 9, 11 and 12) of the Minho River TFWs (Fig. 1) and (ii) an annual sampling program from 2004 to 2010, carried out in October of each year at 16 different sites along the estuarine gradient (Fig. 1). All sites were subtidal with soft sediment substrates, being sites 9, 11 and 12 chosen to assess *P. amnicum* secondary production (Sousa *et al*., 2008b) and because they had the highest densities before the 2005 heat wave.

Abiotic variables were measured at high tide during each site visit. For temperature, conductivity, total dissolved solids, redox potential, salinity, dissolved oxygen and pH measurements were made, *in situ*, close to the bottom, using a multi-parametric sea gauge YSI 6820. Water samples were collected at the surface to assess nitrites, nitrates, ammonia, phosphates and hardness, using colorimetric methods in the laboratory. Sediment samples were collected and analyzed for granulometry and organic matter content, following the methodology described in Sousa *et al*. (2006).

P. amnicum quantitative samples were obtained using a Van Veen grab with an area of 500 cm^2 and a maximum capacity of 5000 cm^3. For the monthly sampling program, six replicates were collected per site (five replicated samples for biological characterization and one for sediment analysis). For the annual sampling program, five replicates were collected per site (four replicated samples for biological characterization and one for sediment analysis), except in 2004 when three replicates were collected (two replicated samples for biological characterization and one for sediment analysis). The biological samples were processed through a 500 μm sieve and *P. amnicum* individuals were separated and measured; all individuals with a shell length less than 4 mm were considered juveniles as described in Araujo *et al*. (1999). *P. amnicum* biomass was calculated using the ash-free dry weight (AFDW) method following Sousa *et al*. (2006).

Data analysis

Information about the monthly cumulative river flow from 1990 onwards was gathered from the Foz do Mouro hydrometric station using data from the Water Institute of Portugal (INAG). We calculated the mean monthly cumulative values along the last 20 years as a proxy of a normal year and differences in the mean annual cumulative river flows for the years 2004–2010 were tested using ANOVA.

Principal component analysis (PCA) was carried out for ordination of sites based on the abiotic factors

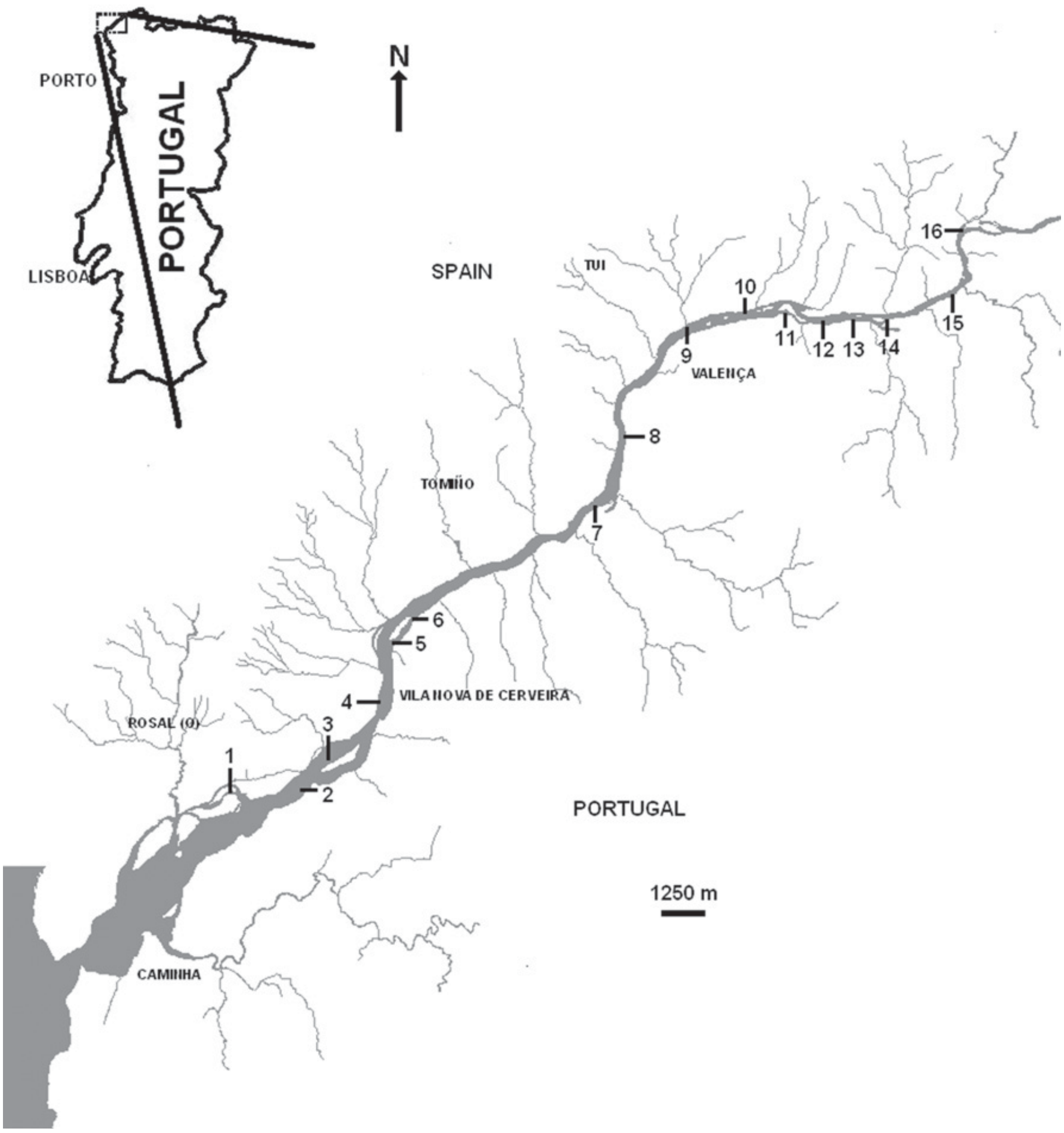

Fig. 1. Map of the Minho River estuary showing the 16 sampling site locations.

measured during the annual sampling (mean values from 2004 to 2010). Prior to the analysis, values were normalized to avoid skew in the dataset. Additionally, an ordination analysis was performed, using group average linkage and Euclidian distance, in order to separate sampling sites into groups with similar environmental characteristics. These analyses were carried out using the PRIMER 6 package (Clarke and Warwick, 2001). Since most data departed from normality (Shapiro–Wilk's *W* test), comparisons in density and biomass of *P. amnicum* along space and time were made using the non-parametric Mann–Whitney *U* test (pairwise) or Kruskal–Wallis (multiple comparisons) using Statistica version 7 (Statsoft, 2004).

Results

Historical data of the cumulative river flows measured at the River Minho from October 2004 to September 2010 plus the mean monthly values of the last 20 years are given in Figure 2. The mean annual cumulative river flow for the years 2004–2010 were clearly distinct ($F = 3.58$; $P < 0.01$). Comparing the cumulative river flow measured during the period comprising October 2004 to September 2010 with the mean values based on data of the last 20 years, we clearly observe a lower cumulative river flow during 2005.

The PCA matrices of abiotic factors *versus* sites (Fig. 3) reveal a clear pattern along the estuarine gradient. From the projection against the first axis of variability (34.0% of the variance explained), sites appear distributed along a physical and chemical gradient, with the sites with finer sediments and high organic matter content (sites 2 and 7–12) along one edge and the sites with coarser sediments and low organic matter content (sites 1, 3–6 and 13–16) located on the other. Along the second axis (23.3% of the variance explained), the distinction of the sites was mostly related to the abiotic factors measured in the water column, mainly conductivity, with sites with higher

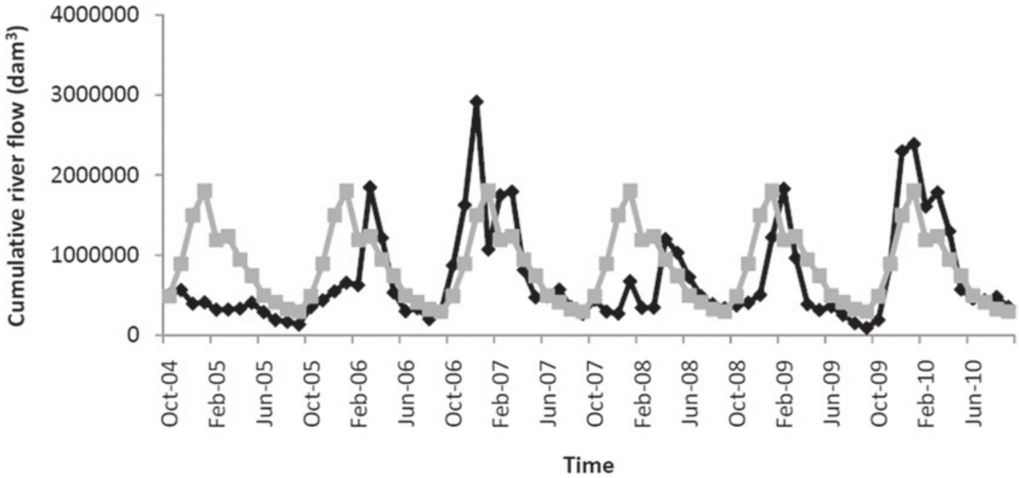

Fig. 2. Cumulative river flows for the Minho River measured at Foz do Mouro hydrometric station. The black line represents cumulative river flows measured between October 2004 and September 2010 and the grey line represents mean values from 1990 to 2010.

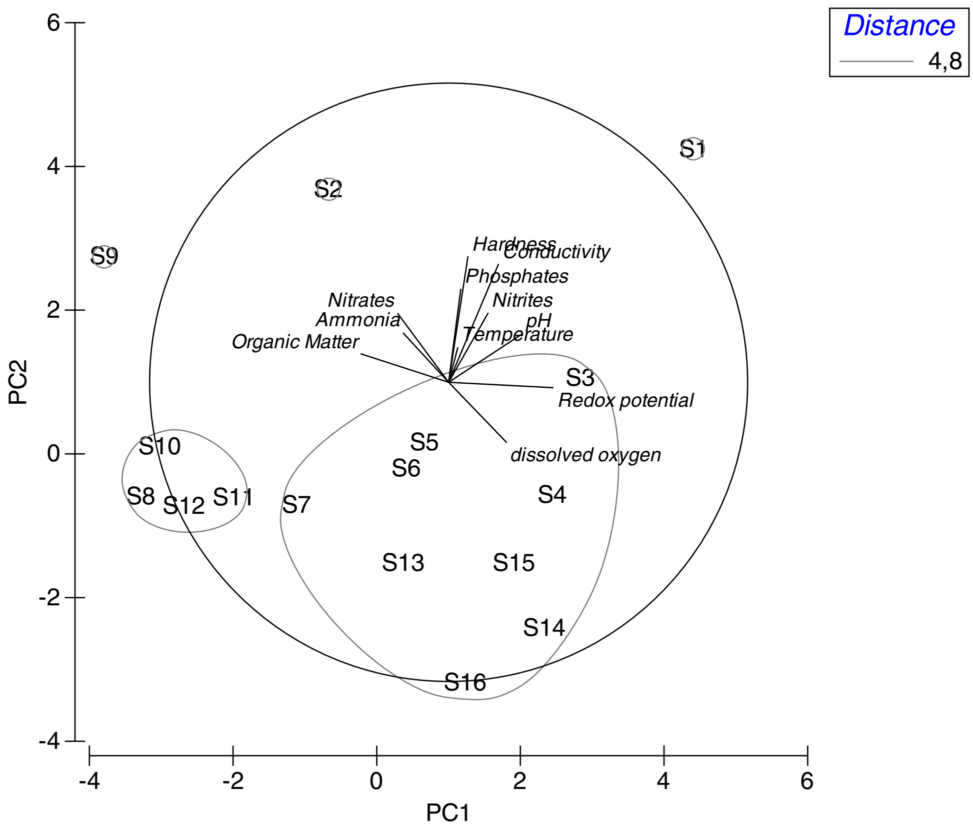

Fig. 3. PCA showing the 16 sampling sites, S1–S16. PC1 explained 34.0% of overall variation and PC2 23.3%. Gray lines indicate the groups found by the cluster analysis (Euclidean distances) of the environmental data from October 2004 to September 2010.

marine influence (sites 1–3) or with higher organic contamination (site 9) along one edge and sites with freshwater characteristics (sites 4–8 and 10–16) located on the other. Detailed data about the abiotic factors for each site are given in Table 1.

There were clear temporal differences in the density ($\chi^2 = 81.24$, $P < 0.01$) and biomass ($\chi^2 = 66.93$, $P < 0.01$) of *P. amnicum* among months (January 2005–August 2006). Additionally, there were significant differences at the spatial scale in density ($\chi^2 = 36.05$, $P < 0.01$) and biomass ($\chi^2 = 67.52$, $P < 0.01$) (Figs. 4(a) and 4(b)). Also, the density and biomass of *P. amnicum* were significantly different when comparing the periods before and after the heat wave (July 2005), being the density ($Z = 7.99$,

Table 1. Measured abiotic factors in each site, for the seven sampled years (mean values plus standard deviation – SD is given). Temperature (T, °C), conductivity (CND, μS.cm^{-1}), total dissolved solids (TDS, mg.L^{-1}), redox potential (ORP, mV), salinity (S, psu), dissolved oxygen (DO, mg.L^{-1}) pH, nitrites (mg.L^{-1}), nitrates (mg.L^{-1}), ammonia (mg.L^{-1}), phosphates (mg.L^{-1}) and hardness (mg.L^{-1}) of water column and organic matter (OM, %), very coarse sand (VCS, %), coarse sand (CS, %), medium sand (MS, %), fine sand (FS, %), very fine sand (VFS, %) and silt + clay (S + C, %) of the sediment.

	S1		S2		S3		S4		S5		S6		S7		S8		S9		S10		S11		S12		S13		S14		S15		S16	
Abiotic factors	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T (°C)	17.2	0.3	17.3	0.3	17.2	0.3	17.1	0.2	17.2	0.3	17.2	0.2	17.2	0.4	17.3	0.4	17.0	0.7	17.2	0.2	17.1	0.2	16.8	0.4	16.8	0.3	16.7	0.4	16.7	0.3	16.4	0.5
CND (μS.cm^{-1})	703.0	540.2	635.5	728.9	209.5	65.4	121.0	17.4	114.8	17.7	188.8	169.5	98.3	6.8	99.3	6.8	183.8	126.6	93.5	16.8	93.0	9.9	82.0	17.8	83.3	17.3	78.8	15.7	78.0	16.1	72.3	18.1
TDS (mg.L^{-1})	0.5	0.4	0.5	0.6	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
ORP (mV)	255.5	109.7	163.0	78.0	168.7	85.4	174.9	78.3	143.3	53.8	180.3	83.3	183.6	74.8	148.2	74.0	117.9	65.7	107.8	50.2	132.0	53.5	156.2	64.5	166.1	60.6	192.9	48.3	214.0	73.9	183.5	64.0
S (psu)	0.5	0.4	0.5	0.5	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DO (mg.L^{-1})	10.5	0.7	9.7	1.2	10.0	1.1	10.1	0.9	10.1	0.4	9.6	0.7	9.5	0.4	9.5	0.7	6.7	2.3	10.0	0.5	10.4	0.3	10.3	0.4	10.3	0.5	10.2	1.1	10.1	0.9	10.0	1.2
pH	8.0	0.5	7.9	0.4	7.8	0.2	7.8	0.3	7.8	0.3	7.5	0.1	7.5	0.1	7.5	0.1	7.4	0.1	7.8	0.2	7.7	0.3	7.7	0.2	7.6	0.3	7.6	0.4	7.7	0.4	7.6	0.4
Nitrites (mg.L^{-1})	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrates (mg.L^{-1})	1.0	0.2	1.5	0.8	1.2	0.5	1.2	0.5	1.4	0.7	1.4	0.4	1.9	0.6	1.3	0.4	1.4	0.5	1.1	0.5	0.8	0.4	0.9	0.4	0.9	0.3	0.7	0.3	1.1	0.7	1.0	0.2
Ammonia (mg.L^{-1})	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.7	1.2	0.1	0.0	0.1	0.0	0.1	0.0	1.5	2.1	0.1	0.0	0.1	0.0	0.1	0.3	0.3	0.7	0.0	0.0	0.1	0.1	0.0	0.0
Phosphates (mg.L^{-1})	0.3	0.4	0.5	0.7	0.3	0.4	0.2	0.3	0.2	0.3	0.4	0.6	0.2	0.2	0.1	0.2	0.5	0.5	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.4	0.2	0.1
Hardness (mg.L^{-1})	46.3	13.8	40.0	7.1	32.5	8.7	30.0	4.1	23.8	8.5	17.5	11.9	22.5	15.0	26.3	16.0	33.8	17.0	25.0	10.8	23.8	12.5	16.3	10.3	31.3	11.1	16.3	4.8	12.5	11.9	12.5	21.8
OM (%)	1.6	0.3	11.3	0.7	0.8	0.2	0.6	0.0	1.3	0.4	1.4	0.8	2.8	0.8	8.5	5.0	5.9	2.0	12.3	1.1	8.9	1.4	16.1	1.4	3.0	2.3	0.6	0.2	2.0	0.5	1.0	0.5
VCS (%)	6.5	2.5	1.1	0.8	5.9	2.8	20.3	17.3	16.2	14.8	5.5	7.4	24.0	26.2	0.2	0.2	0.9	0.4	0.4	0.3	1.9	1.7	3.1	1.2	33.1	25.2	27.6	22.4	51.9	34.3	81.8	13.5
CS (%)	41.7	7.7	9.1	2.8	45.5	8.3	66.1	12.6	28.2	19.3	28.3	23.4	11.3	10.7	1.7	0.3	2.3	0.9	3.1	2.4	7.2	5.5	4.2	1.5	27.7	14.2	50.7	21.2	16.1	8.0	7.4	4.9
MS (%)	44.9	6.3	15.1	1.1	47.3	7.7	12.9	9.3	35.6	12.6	32.7	12.9	11.6	1.6	10.2	2.5	18.2	3.9	12.3	8.3	18.3	9.2	10.1	6.6	16.7	11.3	19.8	8.0	21.0	22.6	6.2	6.0
FS (%)	4.7	2.1	18.6	2.3	1.1	0.6	0.4	0.1	17.4	18.2	22.7	20.0	28.1	21.7	40.3	26.3	42.2	6.9	21.4	7.1	25.0	6.0	18.4	5.8	12.5	12.0	1.4	1.6	7.3	6.1	3.9	4.2
VFS (%)	1.2	0.3	21.3	0.8	0.1	0.1	0.1	0.1	1.9	2.2	9.0	12.7	17.6	11.6	24.1	9.8	18.6	3.5	26.8	2.6	22.1	7.3	30.0	3.7	6.2	5.8	0.3	0.4	2.2	0.7	0.5	0.4
S + C (%)	1.0	0.4	34.8	6.3	0.1	0.2	0.2	0.1	0.7	0.5	1.8	2.1	7.4	3.7	23.4	17.1	17.7	4.2	35.9	11.9	25.5	10.6	34.1	11.9	3.8	4.1	0.1	0.1	1.5	0.9	0.2	0.1

$P < 0.01$) and biomass ($Z = 6.63$, $P < 0.01$) noticeably higher before the heat wave. The maximum density and biomass were 1144 ind.m^{-2} and 5.466 g AFDW.m^{-2}, respectively. Additionally, the number of juveniles was significantly higher in 2005 when compared with 2006 ($Z = 4.53$; $P < 0.01$) (Fig. 5). It is interesting to note that in 2005, juveniles were found from February to July, at particularly high density in May and June, and that in 2006 juveniles were only found from April to June at a very low density relatively to those found in 2005.

Regarding the data collected annually, which covered an extensive area (*ca.* 30 km), it was clear that the species had experienced a significant decline in the density and biomass along the years (Figs. 6(a) and 6(b)). Mean density and biomass values in 2004 reached more than 80 ind.m^{-2} and 0.700 g AFDW.m^{-2}, respectively, but in 2009 and 2010 these values declined to less than 1 ind.m^{-2} and 0.004 g AFDW.m^{-2}, respectively. The maximum density and biomass were 750 ind.m^{-2} and 7.420 g AFDW.m^{-2}, respectively. Moreover, the number of sites colonized by this species substantially decreased along the years, from a maximum of 8 in 2005 and a minimum of 1 in 2009 (Fig. 7). On the other hand, mean density and biomass of the invasive species *Corbicula fluminea*, although suffering a decline in 2005 and 2009, rapidly recover to earlier density and biomass (Figs. 8(a) and 8(b)).

Discussion

The perturbation from more frequent extreme climatic events can have a significant impact on organisms, which often is ecologically more relevant than fluctuations in the mean climate (Stenseth *et al.*, 2002). As in the case of any perturbation, extreme occurrences such as heat waves can result in a sequence of two events: (i) the disturbance marked by the application of the disturbing forces and (ii) the response shown by the biota to the impacts generated by the disturbance (Lake, 2000). The ecological consequences of extreme climatic events have been described as an important threat to freshwater macroinvertebrates since these disturbing events have long been considered important structuring forces that can act at the population, community and ecosystem levels (Lake, 2003; Daufresne *et al.*, 2004, 2007; Mouthon and Daufresne, 2006). Aquatic ecosystems are especially vulnerable to extreme climatic events because these disturbances alter the flow regime that ultimately will affect the biotic component of the ecosystem (Wood and Petts, 1999; Griswold *et al.*, 2008). Looking at the available data on river flow during the last 20 years, we can conclude that the values observed in the Minho River during 2005 were clearly low. The consequences of this low river flow were particularly straightforward during July, when the abiotic changes also included a decrease in the redox potential and dissolved oxygen with reverberating effects on benthic species (Sousa *et al.*, 2008d). These changes were responsible for a significant and extremely rapid decline in *P. amnicum*.

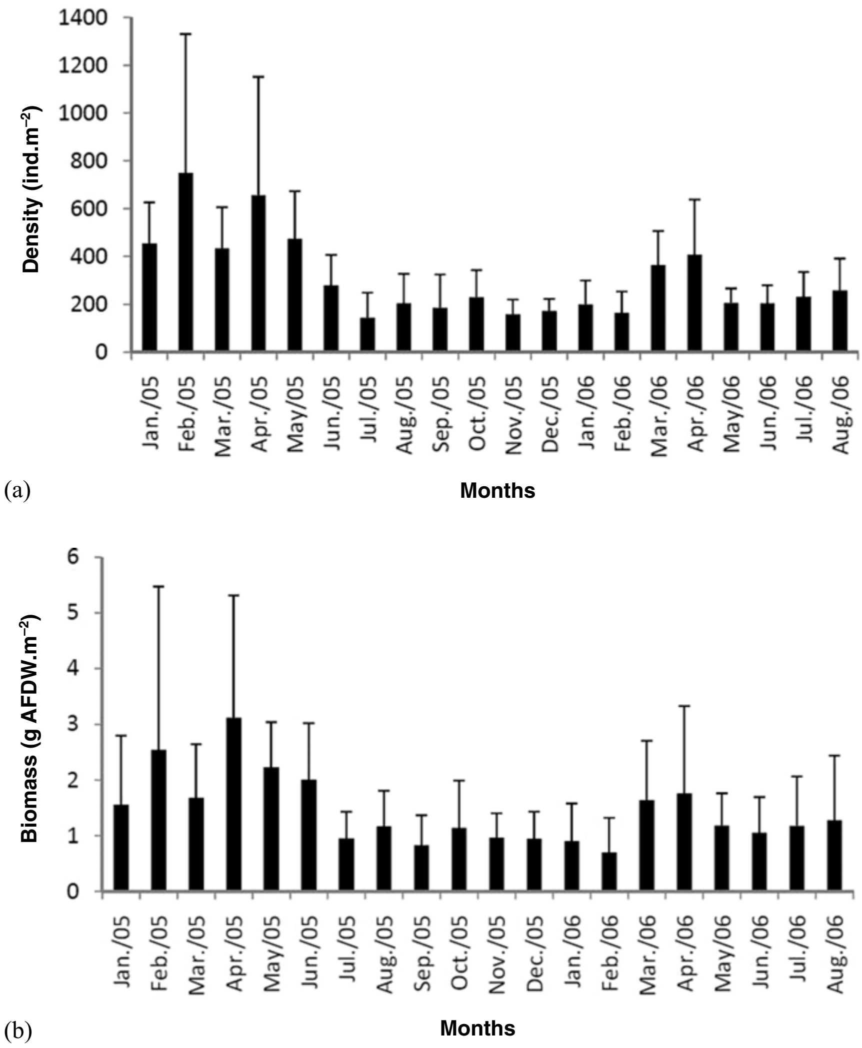

Fig. 4. Mean density (a) and biomass (b) of *P. amnicum* at monthly sampling sites 9, 11 and 12 (data pooled) from January 2005 to August 2006. The confidence bands represent standard deviations.

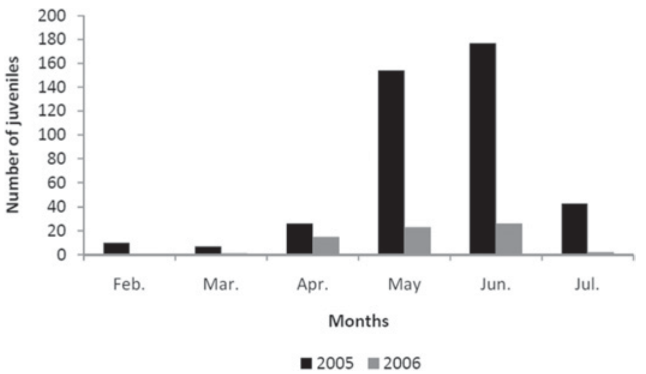

Fig. 5. Total number of juveniles (< 4 mm) collected at monthly sampling sites 9, 11 and 12 (data pooled) from February to July of 2005 and 2006 in the Minho River TFWs.

The reasons for this trend still need to be tested, but it seems that this population did not recover from the impacts generated by the strong 2005 heat wave. Indeed, afterwards this species was not able to reach similar values of density and biomass prior to the heat wave and the spatial distribution suffered a significant reduction, nowadays being restricted to small patches in upstream areas. The effects of heat waves have been responsible for profound and long-lasting effects, resulting in population declines, population bottlenecks and altering the course of the species evolution (Humphries and Baldwin, 2003). *P. amnicum* was significantly affected by the extreme environmental conditions of the 2005 summer (*e.g.* high temperature, very low river flow, low redox potential and low dissolved oxygen) that resulted in extremely high mortality rates of the invasive Asian clam *C. fluminea* (Sousa *et al.*, 2008d; Ilarri *et al.*, 2011). In this lotic system, an extremely high density and biomass of *C. fluminea* exist (Sousa *et al.*, 2008a) and the massive mortality of this invasive species released high amounts of nutrients that in addition to the environmental changes resultant from the heat wave caused massive mortalities of other benthic species, including *P. amnicum* (Sousa *et al.*, 2008d). This situation was especially harsh in sites with fine sediments rich in organic matter, which are the preferential areas

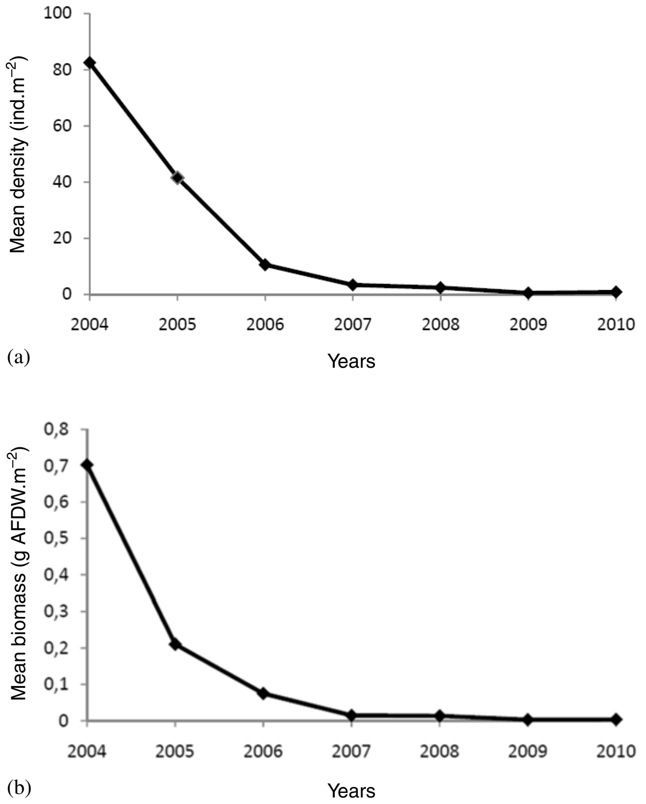

Fig. 6. Mean density (a) and biomass (b) of *P. amnicum* from 2004 to 2010 collected along the 16 different sites in the Minho River TFWs.

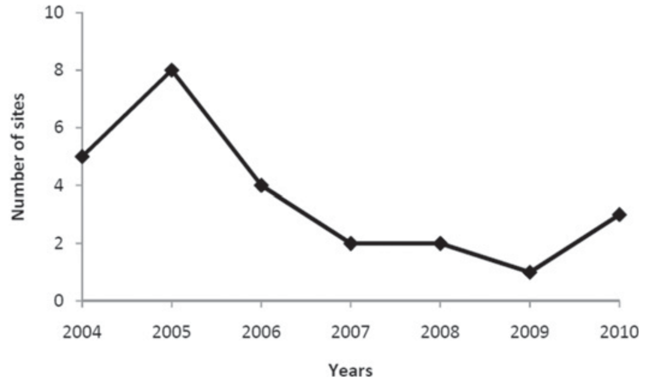

Fig. 7. Number of sites colonized by *P. amnicum* from 2004 to 2010 along the Minho River TFWs.

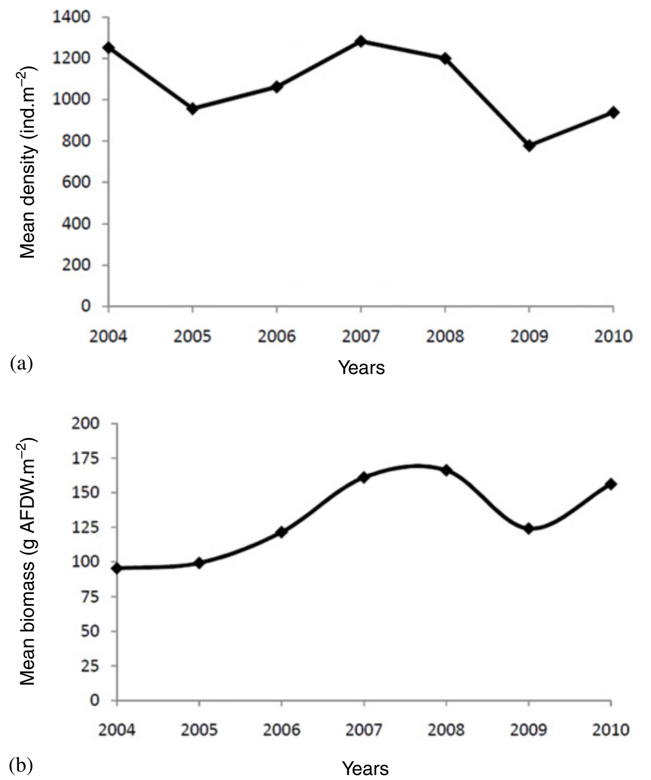

Fig. 8. Mean density (a) and biomass (b) of *C. fluminea* from 2004 to 2010 collected along the 16 different sites in the Minho River TFWs.

for the settlement of *P. amnicum* (Sousa *et al.*, 2008f). However, even facing increasing mortality rates *P. amnicum* still survived after the heat wave, but it has been facing lower or inconspicuous recruitment along the subsequent years. This situation was responsible for an extensive declining trend and until now *P. amnicum* never had a large recruitment and is facing a high risk of extirpation due to prolonged decreases in reproductive output. This failure in recruitment after 2005 was remarkable and we do not possess the full explanation for this fact. It is well established that *P. amnicum* has a very short life cycle (not more than two years in this area; Sousa *et al.*, 2008b) and the low number of juveniles in 2006 was probably an outcome of the lower physiological condition resulted from the heat wave impact and this reduced the capacity to generate a great number of larvae. Another possible explanation could be related to the faster recovery of the invasive species *C. fluminea* that after suffering a great decline in 2005 rapidly increased its density and biomass on the subsequent years (Sousa *et al.*, 2008c; Ilarri *et al.*, 2011). It seems that this invasive species reestablished very quickly, enlarging and increasing its population range and feasibility, thus enhancing its ability to compete with the native species for space and/or food and putting some problems to the recruitment of the native bivalve species.

P. amnicum has been facing a rapid reduction in its spatial distribution through the past three decades. Qualitative data showed that in the 1980s this species colonized all the Minho River TFWs (from sites 1 to 16 of this study); however, after the introduction of *C. fluminea* (1989) the downstream area was the first where *P. amnicum* disappeared (Araujo *et al.*, 1999). In addition to the possible negative interaction between *P. amnicum* and *C. fluminea*, *P. amnicum* seems to be very sensitive to higher conductivity (Sousa *et al.*, 2008f). The construction of several upstream dams over the last four decades and climate change may decrease the flow of fresh water to the estuary, increasing the influence of sea water to upstream areas and consequently intensifying the environmental stress to this native population (Sousa *et al.*, 2008f).

The Minho River functions as the southern European limit of *P. amnicum* distribution and was once the healthiest and largest population inside the Iberian Peninsula (Araujo *et al.*, 1999). The possible extirpation of this species in the Minho River may also mean its disappearance from the Iberian Peninsula. According to Araujo *et al.* (1999) only the Orbigo River (Spain) had a small but stable population in the 1990s; however, this population has not been studied since. More striking is that our study shows a substantial decline in very few years, without any sign of recovery until now. Although *P. amnicum* is a widely distributed species in the North and Central Europe there are already several signs of declines in density and spatial distribution. In a recent study, Mouthon and Daufresne (2008) showed a similar trend as described in this study on the Saône River (France). Indeed, these authors described a massive decline of *P. amnicum* after the strong 2003 heat wave without any sign of recovery in the subsequent years. Similar declines could also be occurring in other European ecosystems caused by extreme climatic events and/or others impacts (*e.g.* pollution, introduction of invasive species, loss and/or degradation of habitat and water regulation). However, due to paucity of recent quantitative studies addressing this question, the current status of *P. amnicum* in Europe is not known.

In conclusion, freshwater molluscs have been facing a great number of threats that have been responsible for high rates of extinctions in the last few decades (Lydeard *et al.*, 2004; Strayer *et al.*, 2004), and some extinctions may be yet unrecognized. This study is an example of an extremely rapid decline over a large spatial area. The recovery of *P. amnicum* after the disturbance imposed by the 2005 heat wave may take considerable time and in this particular case it seems that this species is greatly depleted being the final result the possible extirpation from this river and from the Iberian Peninsula if any conservational measure is taken in account.

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References

Araujo R., Ramos M.A. and Molinet R., 1999. Growth pattern and dynamics of a southern peripheral population of *Pisidium amnicum* (Müller, 1774) (Bivalvia: Sphaeriidae) in Spain. *Malacologia*, *41*, 119–137.

Clarke K.R. and Warwick R.M., 2001. Change in Marine Communities an Approach to Statistical Analysis and Interpretation, Plymouth Marine Laboratory, Plymouth.

Costa-Dias S., Freitas V., Sousa R. and Antunes C., 2010. Factors influencing epibenthic assemblages in the Minho estuary (NW Iberian Peninsula). *Mar. Pollut. Bull.*, *61*, 240–246.

Daufresne M., Roger M.C., Capra H. and Lamouroux N., 2004. Long-term changes with in the invertebrate and fish communities of the Upper Rhône River: effects of climatic factors. *Glob. Change Biol.*, *10*, 124–140.

Daufresne M., Bady P. and Fruget J.F., 2007. Impacts of global changes and extreme hydro-climatic events on macroinvertebrate community structures in the French Rhône River. *Oecologia*, *151*, 544–559.

Dillon R.T. Jr., 2000. The Ecology of Freshwater Mollusks, Cambridge University Press, Cambridge, 509 p.

Dudgeon D., Arthington A.H., Gessner M.O., Kawabata Z., Knowler D.J., Lévêque C., Naiman R.J., Prieur-Richard A., Soto D., Stiassny M.L.J. and Sullivan C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev.*, *81*, 163–182.

Dyduch-Falniowska A., 1983. Age structure of the populations of *Pisidium* species from two localities in southern Poland. *Aquat. Ecol.*, *17*, 111–117.

France R. and Rigg C., 1998. Examination of the "founder effect" in biodiversity research: patterns and imbalances in the published literature. *Divers. Distrib.*, *4*, 77–86.

Griswold M.W., Berzinis R.W., Crisman T.L. and Golladay S.W., 2008. Impacts of climatic stability on the structural and functional aspects of macroinvertebrate communities after severe drought. *Freshw. Biol.*, *53*, 2465–2483.

Higgins J.V., Bryer M.T., Khoury M.L. and Fitzhugh T.W., 2005. A freshwater classification approach for biodiversity conservation plan. *Conserv. Biol.*, *19*, 432–445.

Holopainen I.J., 1979. Population dynamics and production of *Pisidium* species (Bivalvia: Sphaeriidae) in the oligotrophic and mesohumic lake Paajarv, southern Finland. *Arch. Hydrobiol.*, *54*, 446–508.

Humphries P. and Baldwin D.S., 2003. Drought and aquatic ecosystems: an introduction. *Freshw. Biol.*, *48*, 1141–1146.

Ilarri M., Antunes C., Guilhermino L. and Sousa R., 2011. Massive mortality of the Asian clam *Corbicula fluminea* in a highly invaded area. *Biol. Invasions*, *13*, 277–280.

Lake P.S., 2000. Disturbance, patchiness, and diversity in streams. *J. N. Am. Benthol. Soc.*, *19*, 573–592.

Lake P.S., 2003. Ecological effects of perturbation by drought in flowing waters. *Freshw. Biol.*, *48*, 1161–1172.

Lawler J.J., Aukema J.E., Grant J.B., Halpern B.S., Kareiva P., Nelson C.R., Ohleth K., Olden J.D., Schlaepfer M.A., Silliman B.R. and Zaradic P., 2006. Conservation science: a 20-year report card. *Front. Ecol. Environ.*, *4*, 473–480.

Lydeard C., Cowie R.H., Ponder W.F., Bogan A.E., Bouchet P., Clark S.A., Cummings K.S., Frest T.J., Gargominy O., Herbert D.G., Hershler R., Perez K.E., Roth B., Seddon M., Strong E.E. and Thompson F.G., 2004. The global decline of non-marine mollusks. *BioScience*, *54*, 321–330.

Malmqvist B. and Rundle S., 2002. Threats to the running water ecosystems of the world. *Environ. Conserv.*, *29*, 134–153.

Mouthon J. and Daufresne M., 2006. Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saône: a large lowland river and of its two main tributaries (France). *Glob. Change Biol.*, *12*, 441–449.

Mouthon J. and Daufresne M., 2008. Population dynamics and life cycle of *Pisidium amnicum* (Muller) (Bivalvia: Sphaeriidae) and *Valvata piscinalis* (Muller) (Gastropoda: Prosobranchia) in the Saône river, a nine-year study. *Ann. Limnol. - Int. J. Lim.*, *44*, 241–251.

Régnier C., Fontaine B. and Bouchet P., 2009. Not knowing, not recording, not listing: numerous unnoticed mollusk extinctions. *Conserv. Biol.*, *23*, 1214–1221.

Ricciardi A. and Rasmussen J.B., 1999. Extinction rates of North American freshwater fauna. *Conserv. Biol.*, *13*, 1220–1222.

Sala O.E., Chapin F.S., Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L.F., Jackson R.B., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. and Wall D.H., 2000. Global biodiversity scenarios for the year 2100. *Science*, *287*, 1770–1774.

Sousa R., Guilhermino L. and Antunes C., 2005. Molluscan fauna in the freshwater tidal area of the River Minho estuary, NW of Iberian Peninsula. *Ann. Limnol. - Int. J. Lim.*, *41*, 141–147.

Sousa R., Dias S. and Antunes C., 2006. Spatial subtidal macrobenthic distribution in relation to abiotic conditions in the Lima estuary, NW of Portugal. *Hydrobiologia*, *559*, 135–148.

Sousa R., Antunes C. and Guilhermino L., 2007. Species composition and monthly variation of the Molluscan fauna in the freshwater subtidal area of the River Minho estuary. *Estuar. Coast Shelf Sci.*, *75*, 90–100.

Sousa R., Rufino M., Gaspar M., Antunes C. and Guilhermino L., 2008a. Abiotic impacts on spatial and temporal distribution of *Corbicula fluminea* (Müller, 1774) in the River Minho Estuary, Portugal. *Aquat. Conserv.*, *18*, 98–110.

Sousa R., Nogueira A.J.A., Antunes C. and Guilhermino L., 2008b. Growth and production of *Pisidium amnicum* (Müller, 1774) in the freshwater tidal area of the River Minho estuary. *Estuar. Coast Shelf Sci.*, *79*, 467–474.

Sousa R., Dias S., Guilhermino L. and Antunes C., 2008c. River Minho tidal freshwater wetlands: threats to faunal biodiversity. *Aquat. Biol.*, *3*, 237–250.

Sousa R., Nogueira A.J.A., Gaspar M., Antunes C. and Guilhermino L., 2008d. Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774): possible implications for ecosystem functioning. *Estuar. Coast Shelf Sci.*, *80*, 289–295.

Sousa R., Dias S., Freitas V. and Antunes C., 2008e. Subtidal macrozoobenthic assemblages along the River Minho estuarine gradient (north-west Iberian Peninsula). *Aquat. Conserv.*, *18*, 1063–1077.

Sousa R., Morais P., Antunes C. and Guilhermino L., 2008f. Factors affecting *Pisidium amnicum* (Müller, 1774; Bivalvia: Sphaeriidae) distribution in the River Minho estuary: consequences for its conservation. *Estuaries Coasts*, *31*, 1198–1207.

Spooner D.E. and Vaughn C.C., 2006. Context-dependent effects of freshwater mussels on stream benthic communities. *Freshw. Biol.*, *51*, 1016–1024.

StatSoft Inc., 2004. Statistica Version 7 for Windows. http://www.statsoft.com/textbook/stathome.html [Accessed January 3, 2011].

Stenseth N.C., Mysterud A., Ottersen G., Hurrell J.W., Chan K.-S. and Lima M., 2002. Ecological effects of climate fluctuations. *Science*, *297*, 1292–1296.

Strayer D.L. and Dudgeon D., 2010. Freshwater biodiversity conservation: recent progress and future challenges. *J. N. Am. Benthol. Soc.*, *29*, 344–358.

Strayer D.L., Downing J.A., Haag W.R., King T.L., Layzer J.B., Newton T.J. and Nichols S.J., 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience*, *54*, 429–439.

Vaughn C.C. and Spooner D.E., 2006. Unionid mussels influence macroinvertebrate assemblage structure in streams. *J. N. Am. Benthol. Soc.*, *25*, 691–700.

Vörösmarty C.J., McIntyre P.B., Gessner M.O., Dudgeon D., Prusevich A., Green P., Glidden S., Bunn S.E., Sullivan C.A., Liemann C.R. and Davies P.M., 2010. Global threats to human water security and river biodiversity. *Nature*, *467*, 555–561.

Watson A.M. and Ormerod S.J., 2005. The distribution and conservation of threatened Sphaeriidae on British grazing marshland. *Biodivers. Conserv.*, *14*, 2207–2220.

Wood P.J. and Petts G.E., 1999. The influence of drought on chalk stream macroinvertebrates. *Hydrol. Process.*, *13*, 387–399.

Zedler J.B. and Kercher S., 2005. Wetland resources: status, trends, ecosystem services and restorability. *Annu. Rev. Env. Resour.*, *30*, 39–74.

Zettler M., 1996. The aquatic malacofauna (Gastropoda et Bivalvia) in the catchment area of a North German Lowland river, the Warnow. *Limnologica*, *26*, 327–337.

Zettler M.L. and Daunys D., 2007. Long-term macrozoobenthos changes in a shallow boreal lagoon: comparison of a recent biodiversity inventory with historical data. *Limnologica*, *37*, 170–185.