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Freshwater mollusc biodiversity and conservation in two stressed Mediterranean basins

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ABSTRACT

This paper reviews the environmental factors that influence biodiversity of freshwater mollusc communities and conservation status of watercourses in two Mediterranean acid mine drainage-impacted basins of the southern Iberian Peninsula. We found 17 mollusc species: 14 gastropods (10 native and 4 introduced) and 3 bivalves. We found five distribution patterns: native headwater (*Arganiella wolfi*, *Stagnicola palustris*, *Unio delphinus*, *Pisidium casertanum* and *Pisidium personatum*) and mouth (*Hydrobia acuta*, *Peringia ulvae* and *Myosotella myosotis*) sensitive-stenochoric species, intermediate sensitive-widely distributed species (*Planorbarius metidjensis* and *Radix balthica*), insensitive-eurychoric species (*Ancylus fluviatilis*), and erratic-distribution pattern species (*Galba truncatula* and *Planorbis carinatus*). The highest biodiversity indices have been found in non-impacted headwaters and, to a lesser extent, in tidal streams. The biodiversity of the middle reaches, with varying degrees of impact by acid mine drainage and high water deficit, was scarce and dominated by introduced species. Over 30% of the variation in native and introduced species richness is explained by environmental gradients related to heterogeneity (instream macrophytes cover and Fhi and Qbr indices) and acid runoffs (pH, conductivity, turbidity and concentration of sulphides). Severely impacted sites have no mollusc species. The conservation status of watercourses is also very remarkably influenced by the heterogeneity and contamination of the environment. Conservation values are higher in water bodies located in protected northern and southern sites in both basins.

Introduction

The composition and spatial relationships of aquatic communities are related to the habitat structure and variation of environmental factors along complex gradients from sources to mouth (Heino 2000; Lorenz et al. 2004). Freshwater biodiversity patterns are closely related to local geographic features and physicochemical biotope structure, together with biological interactions and historical and random factors (Vannote et al. 1980; Palmer 1999; Malm et al. 2005; Hoeinghaus et al. 2007). Such ecological conditions are the primary factors controlling the composition and microdistribution of freshwater benthic macroinvertebrates, producing a river zonation based on changing local assemblage composition along longitudinal gradients (Vannote et al. 1980; Usseglio-Polatera and Beisel 2002; Hoeinghaus et al. 2007).

Freshwater ecosystems have been subjected to a wide range of human impacts (Ricciardi and Rasmussen 1999; Lydeard et al. 2004; Dudgeon et al. 2006). The anthropogenic global changes in freshwater communities highlight the urgent need to understand the degree of disturbance of these ecosystems and how these changes affect freshwater iota. In this sense, the Water Framework Directive (hereafter WFD) (Directive 2000/60/EC) requires that water resources be subject to ecological assessment, to provide a basis for the management and restoration of catchments. As bioindicators of the integrity and/or degradation of inland waters, the knowledge of distribution patterns, spatial relationships and habitat use of the freshwater biota is of capital importance from a conservationist perspective.

Biotic stress and resilience of Mediterranean freshwater macroinvertebrates affected by acid mine drainage (hereafter AMD) depends on two periodic phenomena linked to seasonality of low discharge and summer drought (Resh et al. 1996; Pires et al. 2000; Everard and Powell 2002; Irvine 2004; Bonada et al. 2006) and on local allochthonous inputs related to mine water discharges and oxidation and hydrolysis of metal sulphide deposits (Nieto et al. 2007; Sarmiento et al. 2009). These alterations originate a highly restrictive environment characterised by increased concentrations of dissolved heavy metals, high conductivity and low pH levels in affected zones (Olías et al. 2004; Cánovas et al. 2007; Nieto

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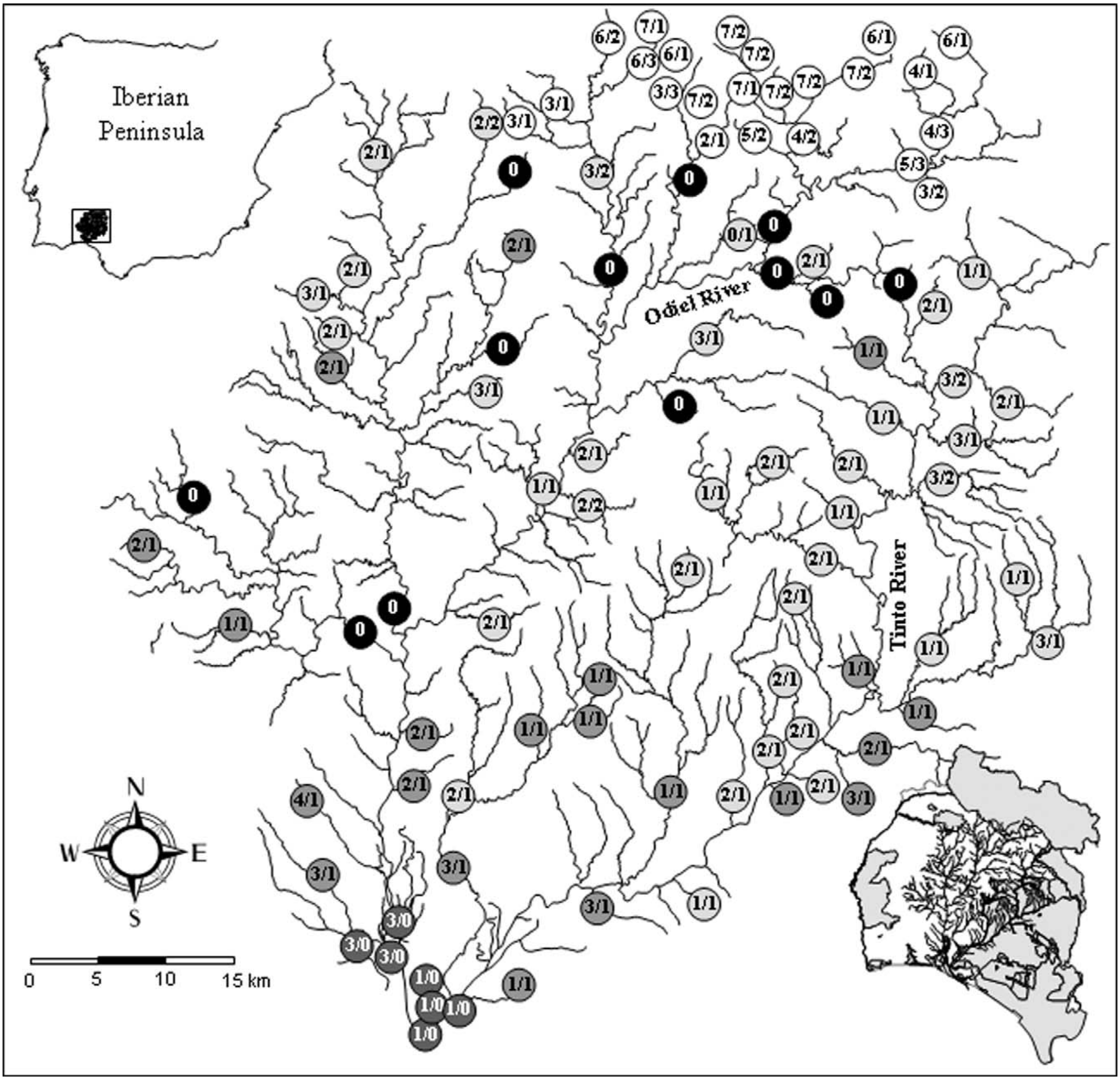

Fig. 1. Location of the study area. The sampling sites are represented by circles, black (severely impacted sites, Si), dark grey (Ti sites), medium grey (S1 sites), pale grey (S2 sites), white (minimally impacted sites, Ni) (see *Materials and methods* and Fig. 2). At each site appears the number of native species/number of introduced species of freshwater molluscs. In the right bottom is the map of the Natura 2000 Network (in grey) in the vicinity of the basins of Odiel and Tinto.

et al. 2007) that influence the distribution of aquatic biota by the appearance of barriers to migration (Cain et al. 2004; Gerhardt et al. 2004). Decreased acid-neutralising capacity contributes to maximise the biotic stress of benthic macroinvertebrate communities inhabiting those polluted Mediterranean streams (Driscoll et al. 2003; Bowman et al. 2006; Tripole et al. 2006). As a consequence, macroscopic flora and fauna do not exist in extremely polluted environments (Amaral-Zettler et al. 2003) and invertebrate biocoenoses are confined to unpolluted, or less polluted, tributaries or sub-basins.

In semi-arid Mediterranean freshwater environments affected by AMD, the main threats to the conservation of communities come from the strong synergistic stress caused by the great variability in water availability coupled with the presence of acid runoffs (Graça et al. 1989; De Nicola and Stapleton 2002; Trouve et al. 2003; Bonada et al. 2006; Bowman et al. 2006; Mouthon and Daufresne 2006). Furthermore, the presence of invasive species is another disturbing pressure generating considerable conservation problems (Ricciardi and Rasmussen 1999; Dudgeon et al. 2006). Introduced species are widespread and expanding their ranges (Loo et al. 2007; Ricciardi 2007) and compete with native species for food and space (Ortiz and Puig 2007; Zaiko et al. 2007).

This work reviews the distribution of freshwater mollusc fauna and conservation status of watercourses in Mediterranean AMD-impacted basins of the southern Iberian Peninsula. Hence, the study aims to: (1) analyse the distribution of species, native and introduced; (2) search for patterns in the distribution of different biodiversity indices (species richness, Shannon-Weiner's diversity, endemicity and faunistic originality); and (3) evaluate the impact of environmental variables on the conservation value of the studied watercourses. The results could be a useful tool with regard to future conservation and restoration strategies and can be integrated into monitoring and bioassessment programmes through biotic indices, ensuring objective evaluations.

Materials and methods

Study area

This study was conducted in the basins of the rivers Odiel and Tinto, both in the southwest of the Iberian Peninsula, in the biogeographic Ecoregion 1 (Illies 1978; Annex XI of the WFD). Both watersheds flow south through the Iberian Pyrite Belt, the world's largest massive complex of sulphide mineral ores (Buckby et al. 2003; Olías et al. 2004), and drain into the Atlantic Ocean (Fig. 1).

Both basins show different degrees of AMD impact along a head-mouth gradient (López-Archilla and Amils 1999; Olías et al. 2004; Sainz et al. 2005). Middle stretches and mouth of the Odiel River basin and the Tinto River basin as a whole have gradients of heavy metals in solution and moderate to extremely low pH as a result of mining activities and the metabolism of acidophilic-chemolithotrophic microorganisms (López-Archilla and Amils 1999; Amaral-Zettler et al. 2003; González-Toril et al. 2003).

The climate is Mediterranean Pluviseasonal-Oceanic. Most rainfall occurs between October and March and during summer months most streams and tributaries become intermittent or dry. Headwater sites with high rainfall and relatively low temperatures belong to the upper and low Mesomediterranean subhumid bioclimatic belts. Mouth areas belong to the upper thermo-Mediterranean dry bioclimatic belt (Worldwide Bio-Climatic Classification System).

Characterisation of freshwater mollusc community and habitat features

The distribution of freshwater malacofauna and habitat features was compiled from 101 watercourses (59 from the Odiel River basin and 42 from the Tinto River basin), during spring-summer in 2004 and 2005 (for the analysis we used the average values of species richness in both years). The sampling points were selected for their accessibility and sampling was conducted at sites never deeper than 150 cm. Along the embankments of each site, two quadrats (25 m^2, according to the width of the stream) were randomly located along a 20-m transect. Molluscs were sampled within each quadrat using a 20 cm diameter dip-net (250-μm mesh size) for a 20-min period, covering all microhabitats visually detected. Mud and sand particles were also removed (upper 5 cm layer) and filtered with sieves. In stony bottoms and in in-stream vegetation, sampling was conducted by manually brushing molluscs from the rock and plant surface into the net (the surface of the rocks was not added to the surface of the sampled area). The abundance of each species was expressed as number of individuals per m^2 and all molluscs were identified to species level and then returned to the water. Dubious species were determined using specific keys and subsequently preserved in 70% ethanol and deposited in the collections of the Department of Environmental Biology and Public Health, University of Huelva, Spain. Number of native and introduced species was recorded for each site.

To examine the effects of environmental factors on biodiversity metrics, 22 habitat variables were measured or estimated at each sampling point. Variables were grouped into five categories: climatic, geomorphological, hydrological, heterogeneity and physicochemical characteristics. Two approaches were used: 15 *in situ* measures (within each quadrat), which described micro and mesohabitat characteristics at each site, and 7 remote, *ex situ*, measures from GIS and web information (Worldwide Bio-Climatic Classification System) (Table 1).

Table 1
Mean ± standard deviation and ranges of the environmental features used to characterise the sampled sites (underlined: *in situ* measures). 1: Worldwide Bio-Climatic Classification System; 2: Strahler's method (Gordon et al. 2004); 3: Pozuelo et al. (2005); 4: 1, streams always dried during the drought period, 2, streams always dried during the drought period but with isolated pools, 3, streams dried only during severe droughts, 4, streams always with permanent flow; 5: Pardo et al. (2002); 6: Munné et al. (2003). *: effluent characteristics and climate and environment variables potentially perturbed by humans.

	Mean ± sd	Range
Climatic		
Air temperature* (At, 1)	17.2 ± 1.5	14.8–19.2
Precipitation* (Pr, 1)	839.9 ± 223.3	461.0–1126.0
Water surplus* (Ws, 1)	379.4 ± 200.9	66.0–627.0
Water deficit* (Wf, 1)	433.3 ± 83.4	312.0–555.0
Geomorphological		
Altitude (Al, m)	367.4 ± 227.3	18.7–959.7
Order (Or, 2)	1.8 ± 0.9	1–5
Slope (Sl, 3)	2.0 ± 0.7	1–3
Distance to the mouth (Dm, Km)	49.3 ± 22.1	1.4–83.4
Hydrological		
Permanency* (Pe, 4)	2.3 ± 1.2	1–4
Water depth (Wd, cm)	72.8 ± 20.5	25.0–130.0
Channel width (Cw, cm)	400.2 ± 176.5	150.0–1300.0
Heterogeneity		
Boulder (Bo, %)	33.0 ± 16.9	0–55.0
Cobble (Co, %)	25.2 ± 8.7	1.0–40.0
Gravel (Gr, %)	19.9 ± 8.7	5.0–40.0
Sand and limes (Sl, %)	21.9 ± 15.5	5.0–79.0
Instream macrophytes cover* (Ic, %)	16.0 ± 13.0	0–50.0
Fluvial heterogeneity index* (Fhi,5)	38.6 ± 7.9	18.0–48.0
Quality of riparian habitat* (Qbr, 6)	16.2 ± 12.9	0–45.0
Physicochemical		
pH* (pH units)	6.7 ± 1.4	2.7–8.5
Conductivity* (Cn, $\mu S \times cm^{-1}$)	790.5 ± 1540.8	115.0–12380.0
Turbidity* (Tu, $mg \times l^{-1}$)	342.4 ± 185.8	80.0–850.0
SO_4^{2-}* (Su, $mg \times l^{-1}$)	405.3 ± 1882.1	0.05–16818.0

Biodiversity metrics

Four different evaluations of native species biodiversity were made: (1) Species richness (native and introduced species), (2) Shannon-Weiner's diversity index (H') obtained using base-e logarithm, (3) Endemicity index (En), as the inverse of the number of sites in which a species is present (Hessen and Walseng 2008), and (4) Faunistic originality index (Ifo) (Boix et al. 2008).

The conservation value (Cv) of each watercourse, in relation to the communities of freshwater molluscs, was calculated as the sum of the values of species richness, diversity, endemicity and faunal originality of each of them.

Statistical analyses

To summarise environmental complexity, a stream *vs.* environmental data matrix was constructed and used to perform a Principal Component Analysis (PCA) after elimination of auto-correlated variables ($R \geq 0.80$). Axial ecological gradients (PC1 and PC2) were identified correlating each environmental variable to the first two dimensions (Pearson's correlation). The scores of this correlation indicate how well each environmental variable explains the position of the sampled points along the ordination axis. As a summary of the environmental gradient along the studied zone, the habitat conditions were assimilated into the dimension with the highest explanatory power (PC1, approximately 37% of the explained variance, see Table 2). In the analysis of habitat condition have been considered only variables with correlation >0.60 with the first two dimensions of the PCA (Factor Loadings).

To perform a non-hierarchical classification of sites, PC1 was split into five equivalent portions to define groups of sites with similar environmental characteristics (hereafter habitat classes: Si, Ti, S1 and S2 and Ni). To test for significant differences, so defined

Table 2
Multivariate analysis used to define environmental and freshwater mollusc community gradients. In the PCA analysis only loadings >0.60 are shown. Variable codes given in Table 1.

Technique	Extracted gradients	% explained variance (eigenvalue)	Negative extreme	Positive extreme	Denomination
PCA	PC1	36.8 (8.8)	Cn (−0.79)	Fhi (0.83)	Natural-disturbed gradient
			Tu (−0.75)	Pr (0.77)	
			So_4^{2-} (−0.70)	Dm (0.77)	
			Wf (−0.68)	Al (0.77)	
			At (−0.66)	Ic (0.75)	
				Qbr (0.74)	
	PC2	19.2 (4.6)	pH (−0.75)		Acidity gradient
CA	Dim. 1	53.2 (0.9)			Species gradient

habitat classes were compared, in terms of their environmental features, biodiversity and conservation value, by one-way Analysis of variance (ANOVA), with post hoc Tukey's HSD test for paired comparisons.

As freshwater mollusc communities tend to vary along natural longitudinal gradients (Mouthon 1999; Pérez-Quintero 2007), their spatial location within these gradients must be accounted for by species sensitivity-tolerance analyses. A correspondence analysis (CA) was performed in a species presence–absence × sites matrix to identify the main patterns of variation in freshwater mollusc community composition within the study area.

The effect of the presence or absence of species in relation to variables directly related to acid drainage, with the aim of identifying significant differences between occupied and unoccupied sites, was tested using ANOVA analysis.

To model important environmental variables determining mollusc biodiversity, linear regressions were performed between each variable and all the biodiversity and conservation metrics.

Prior to all the statistical analyses, normal distributions were investigated using the Kolmogorov-Smirnov test. If necessary, appropriate transformations $\ln(x+1)$ (continuous variables) or arcsine (percentages) were used to improve normality and remove heteroscedascity. Analyses were performed using SPSS, version 17.0.0.

Results

Habitat characterisation

The first two dimensions of the PCA explained 56.0% of the original variance of the environmental variables (Table 2). PC1 (36.8% of the variance) is mainly related to variables describing natural and disturbed gradients resulted for AMD and water deficit. The negative end of the gradient is represented by physicochemical (conductivity, turbidity, sulphides) and climatic (water deficit, air temperature) stress variables. The positive end of the gradient is dominated by environmental features representing the habitat variability (fluvial heterogeneity, in-stream macrophytes cover, quality of riparian habitat), geomorphology (distance to the mouth, altitude) and climate (precipitation). The gradient described by PC2 (19.2% of the variance) is basically related to the pH of the water courses. Thus, two independent gradients were identified: a natural-disturbed gradient related to PC1 and an acidity gradient related to PC2 (Table 2). All the habitat classes into which PC1 was divided differ markedly in the environmental parameters (ANOVA test $F_{(4,96)}$ between 30.8 and 115.4, $P<0.001$ in all cases; Fig. 2).

Species distribution and sensitivity to AMD-related features

Any species was found in the sampling sites severely impacted by AMD (Si habitat class). In both basins 48 (Odiel) and 41 (Tinto) sampling points were found with species richness ≥1, of which 95.7% of sites (Odiel basin) and 92.7% (Tinto basin) contain introduced species. Altogether, 4663 individuals belonging to 17 species (14 gastropods, 10 native and 4 introduced, and 3 bivalves) were identified. The first dimension of the CA, which accounted for 53.2% of the freshwater mollusc community variance (Table 2), was strongly correlated to both natural-perturbed and acidity gradients (Pearson's $R=-0.65$ and -0.41, respectively, $P<0.001$ in both cases), showing a clear negative spatial distribution in mollusc community composition according to, mainly, acid drainage and water deficit stressors.

The available-used analysis through the five equivalent portions of the natural-perturbed gradient shows the sensitivity of each species to this gradient (Fig. 3). Not considering the severely impacted habitat class Si, the different species follow five defined patterns: (1) sensitive stenochoric headwater species, characterised by the overuse of the best preserved portions (Ni) (native *Arganiella wolfi*, *Stagnicola palustris*, *Unio delphinus*, *Pisidium casertanum* and *Pisidium personatum*, and introduced *Potamopyrgus antipodarum* and *Gyraulus chinensis*); (2) sensitive stenochoric mouth species, inhabiting environments with tidal influence (Ti) (*Hydrobia acuta*, *Peringia ulvae* and *Myosotella myosotis*); (3) intermediate sensitive-widely distributed species, present in 3 portions and over 30 sites, using the best preserved portions and under-using minimally impacted environments (native *Planorbarius metidjensis* and *Radix balthica*); (4) insensitive-eurychoric species, present in 4 portions and over 80 sites, characterised by using the portions as available (native *Ancylus fluviatilis* and introduced *Physella acuta*); and (5) erratic-distribution pattern species (native *Galba truncatula* and *Planorbis carinatus*, and introduced *Ferrissia fragilis*).

The ANOVA analysis divides the native species present in more than 10 sites into two groups (Fig. 4): (1) species with a clear sensitivity, over-using less contaminated environments (*A. wolfi*, *P. casertanum*, *G. truncatula* and *R. balthica*), $P<0.05$ in all cases; and (2) intermediate sensitive (*Planorbarius metidjensis*) and insensitive species (*Ancylus fluviatilis*) are the most tolerant group of species to drastic decreases in pH and increased concentrations of sulphides.

Native freshwater mollusc biodiversity

Biodiversity indices were highly correlated among one another ($P<0.001$ in all cases, except for En-Fo, $P<0.05$). Native species richness, diversity and faunistic originality were significantly related to the 12 environmental gradients defined by PC1 and PC2 (see Table 2). In contrast, endemicity was only related to climatic (air temperature and water deficit) and physicochemical stressors (pH, turbidity and concentration of sulphides) (Table 3). Ten to 62% of the variation in native species richness, 12–38% in Shannon-Weiner's diversity, 5–16% in endemicity and 15–76% in faunistic originality is explained by changes in the environmental features (see Table 3).

All the biodiversity measures varied strongly along environmental gradients. According to their high pollution characteristics, all sites of the Si habitat class are characterised by the absence of macroscopic life. Slightly impacted S1 and S2 sites, composed

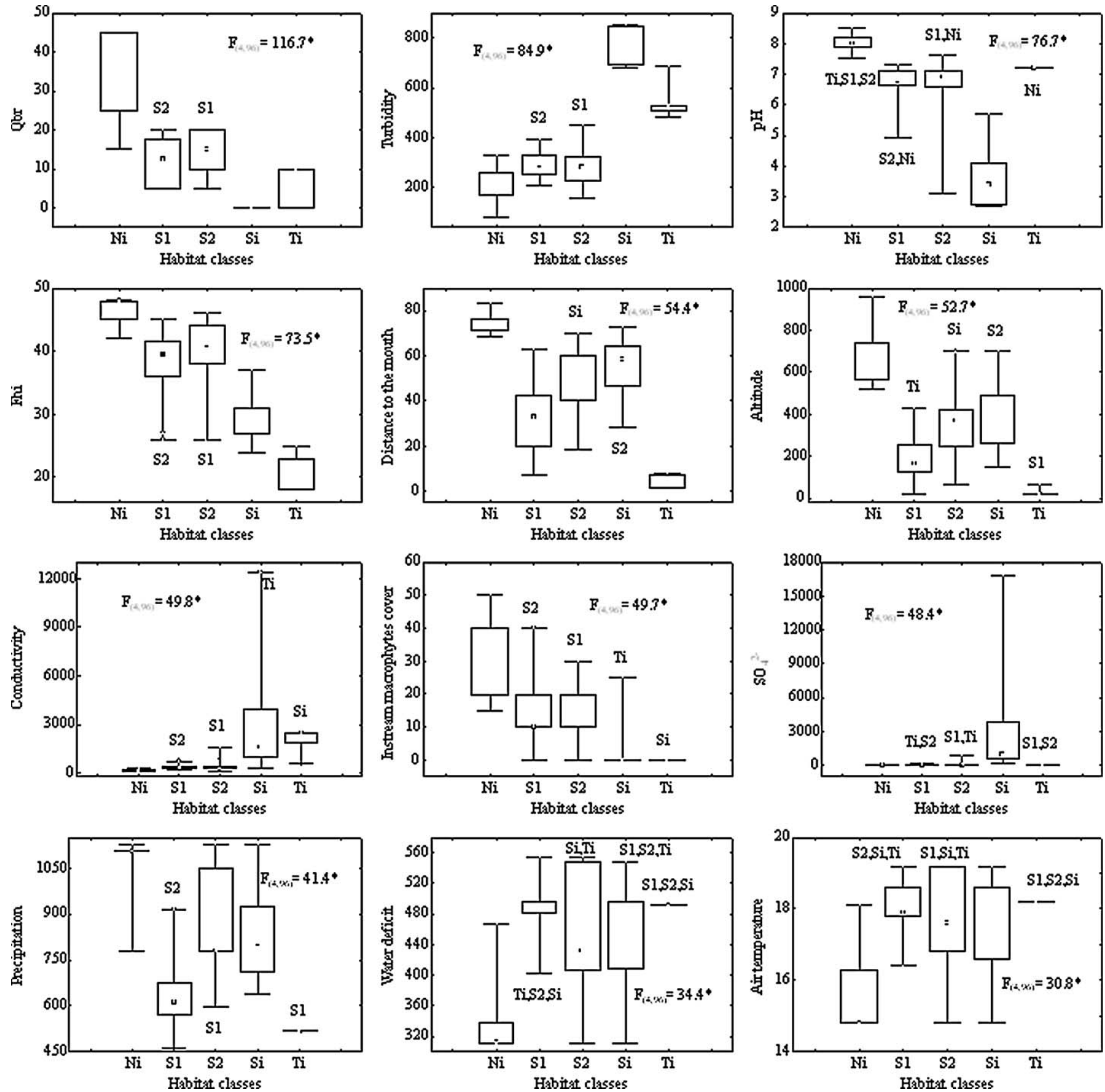

Fig. 2. Differences in the environmental variables with PC1 and PC2 loadings >0.60 (Table 2) across all habitat classes. Ni, minimally impacted sites, S1 and S2, slightly impacted sites, Si, severely impacted sites, Ti, tidal-influenced sites (see *Materials and methods*). $^*P < 0.001$, no significant differences (Tukey's HSD test for paired comparisons) are shown above or below each box. Quadrates, medians, box, percentiles (coefficient = 25), whiskers, the highest and lowest values excluding outliers, circles, outliers (coefficient = 1.5).

exclusively of ephemeral water courses, have poor to moderate biodiversity indices. In contrast, no impacted sites, class Ni, mainly semi-permanent or permanent water courses, have, in general, the highest biodiversity values (Fig. 5).

Introduced species and conservation

The greatest number of introduced species is in non-impacted sites (Fig. 5). The regression analysis using introduced species richness and conservation value as dependent variables shows that these indices are strongly influenced by the environmental features (Table 4). More than 30% of the variation in Isr is explained by physicochemical (turbidity, conductivity, concentration of sulphides and pH) and heterogeneity features (quality of riparian forest, fluvial heterogeneity and in-stream cover). Both native and introduced species numbers increase and decrease along environmental gradients, with significant statistical relationships between both biodiversity indices ($R = 0.46$, $P < 0.0001$). So, abiotic factors would be influential in the establishment of introduced species in AMD-affected Mediterranean water courses. Cv is mainly conditioned ($R^2 \geq 0.30$) by environmental stressors related to the acid drainage (Su, pH, Tu and Cn) and environmental heterogeneity (QBR, Ic and FHI) (Table 4). Cv is also correlated positively with all the biodiversity indices ($P < 0.01$ in all cases). The higher values

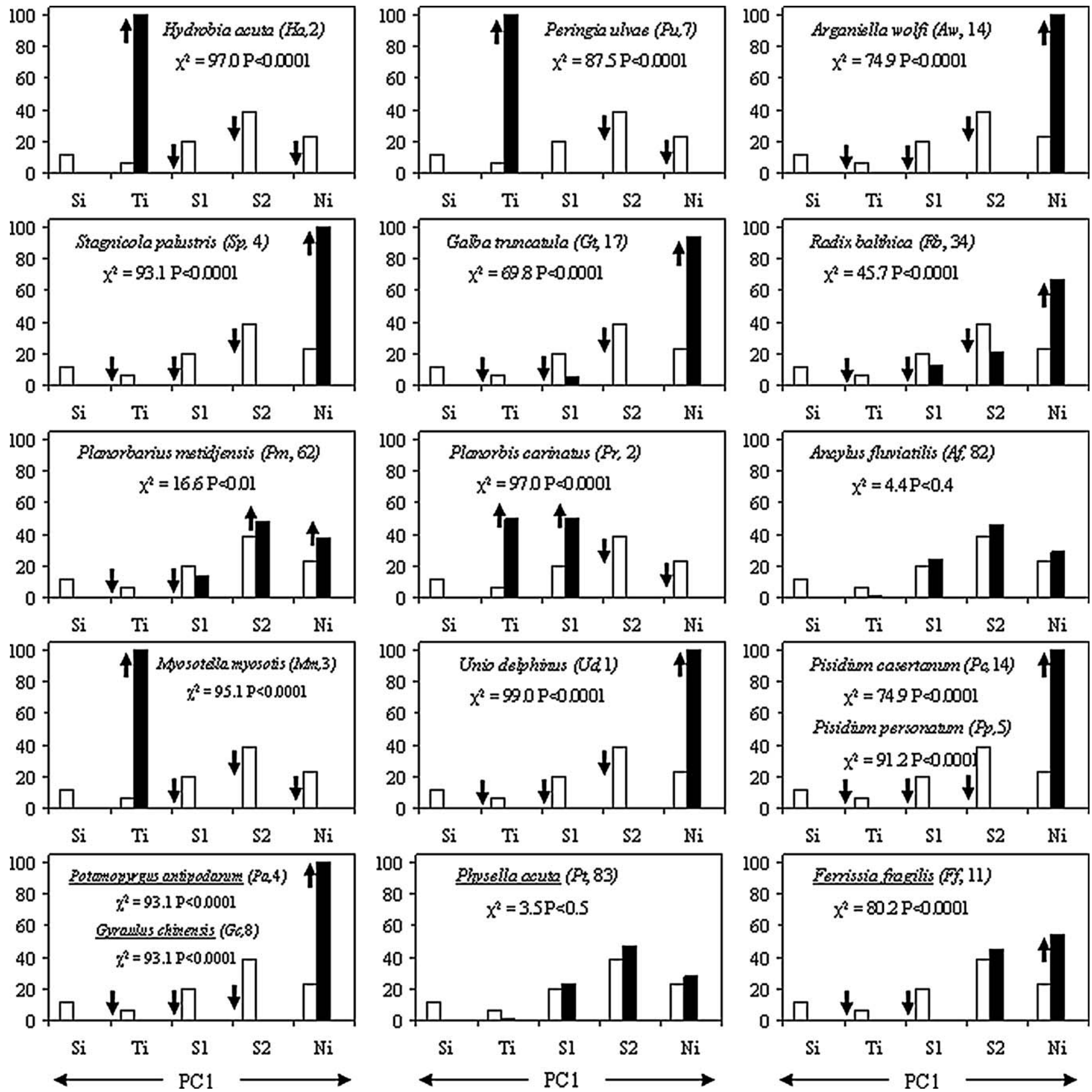

Fig. 3. Preference for the five equivalent portions in which the PC1 gradient was split. The available number of sites is represented in white columns and the number of used in black columns (data are in percentage). The chi-square statistic and its associated *P*-value are also given. Significant differences were interpreted as over-use (up arrows) or under-use (down arrows). Introduced species are underlined. Between parentheses, specie's code and number of sites in which the species is present.

of Cv (on average 6.8 ± 2.2 Sd) are located in well preserved source streams, not impacted by AMD sites (Ni habitat class, average native species richness: 5.3 ± 1.7 Sd) (Fig. 5), with species present only in almost pristine sites at altitudes over 500 m (native sensitive species such as *A. wolfi*, *S. palustris*, *U. delphinus*, *P. casertanum* and *P. personatum*). The lowest values of Cv (2.2 ± 1.2 Sd and 2.3 ± 0.8 Sd, respectively), obviating the severely impacted sites (Si), are located in streams with differing degrees of impact by AMD and water stress (S1 and S2 sites, average native species richness: 1.8 ± 0.9 Sd and 1.9 ± 0.7 Sd, respectively).

Superposing the drainage surface of both Odiel and Tinto River basins with the current Nature 2000 Network of Protected Natural Spaces and Sites of Community Importance (EU Council Directive 92/43/EEC) (see Fig. 1), a high degree of overlap was detected between the water courses with higher native species richness and protected areas not impacted by AMD or water stress (Ni sites). In the remaining sites of community importance (see Fig. 1), the impacts of AMD, water deficit and/or tidal influence decrease the species richness and therefore the conservation value of watercourses.

Discussion

The Water Framework Directive urges the knowledge of the ecological status of the European inland water bodies using biological, physicochemical and hydromorphological indicators (Directive 2000/60/EC, Annex V). Although the use of bioindica-

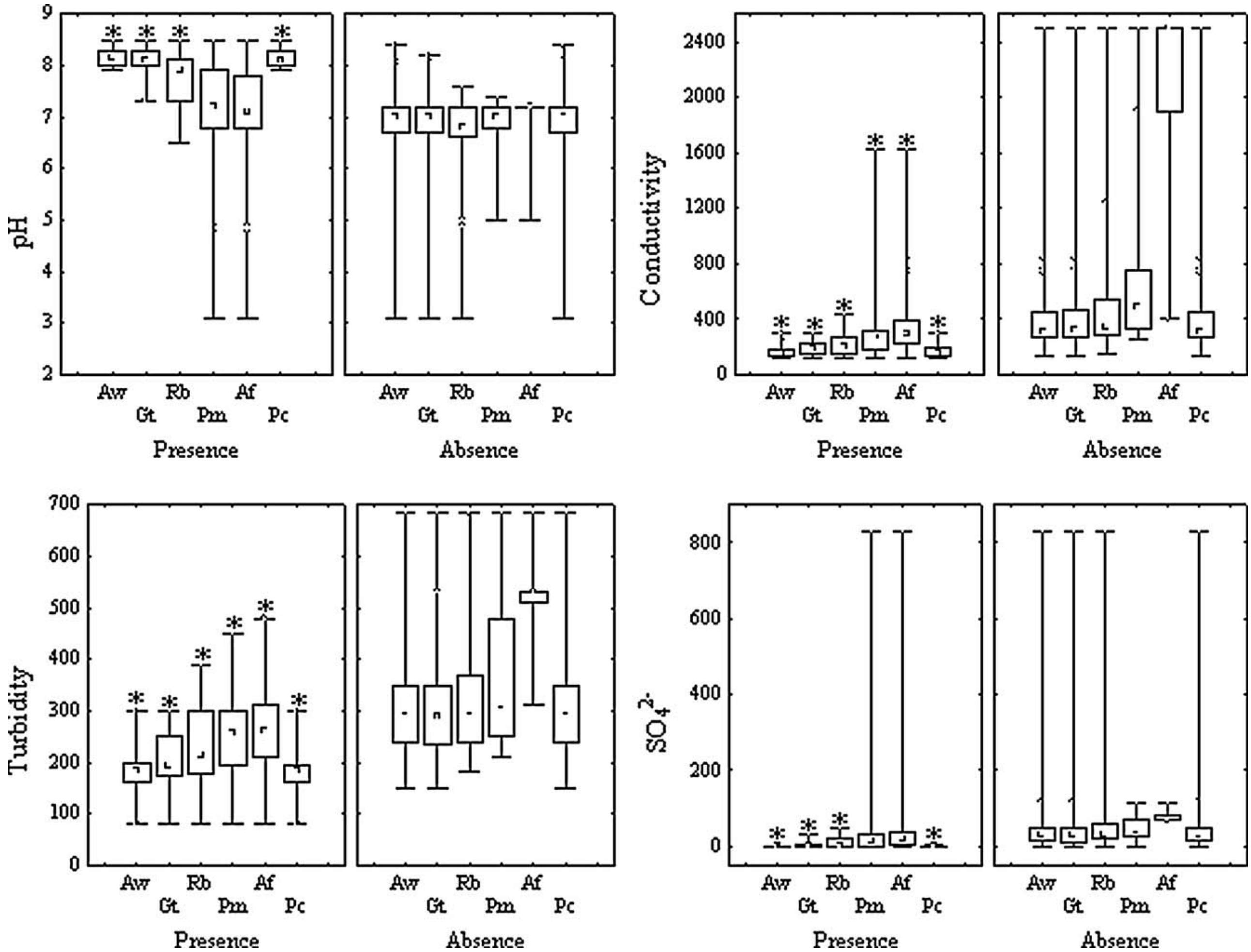

Fig. 4. Presence (left) and absence (right) of native freshwater molluscs in the four environmental gradients related to AMD features (data of the severely stressed habitat class Si have not been considered). Only native species present in, at least, 10 sites have been considered. Quadrates, medians, box, percentiles (coefficient = 25), whiskers, the highest and lowest values excluding outliers, circles, outliers (coefficient, 1.5). *Significant differences ($P < 0.05$) in the ANOVA analysis. Species code in Table 3.

tors is widespread, most studies focussing on freshwater animal communities use fish and benthic arthropods as indicator species (e.g. Iliopoulou et al. 2003; Gerhardt et al. 2004; Hering et al. 2006; Prenda et al. 2006) and little attention has been devoted to freshwater molluscs. The empirical approach developed in this study aims to objectively evaluate the sensitivity/tolerance of freshwater molluscs to provide a useful tool for future conservation and management programmes.

For the implementation of the WFD, species-level data are required at two different scales: presence–absence and abundance data. Although abundance estimates of the studied species are available (used for calculating the Shannon-Weiner's diversity index), to analyse their sensitivity we preferred to use presence–absence data in order to provide a simple approximation regardless of the characterisation of species abundance, much more laborious than the simple assessment of presence–absence.

Table 3

Regression models for the relationships between native species richness (Nsr), diversity (H'), endemicity (En) and faunistic originality (Fo) and environmental variables with PC1 and PC2 loadings > 0.60 (see Table 2). R^2: adjusted R^2, β: standardized regression coefficient, 1: $P < 0.05$, 2: $P < 0.01$, 3: $P < 0.001$, ns: no significant.

	Nsr			H'			En			Fo		
	$F_{(1,99)}$	R^2	β	$F_{(1,99)}$	R^2	β	$F_{(1,99)}$	R^2	β	$F_{(1,99)}$	R^2	β
At	27.4^3	0.22	−0.47	48.7^3	0.33	−0.57	13.8^3	0.12	−0.35	17.7^3	0.15	−0.39
Pr	11.6^3	0.10	0.32	14.2^3	0.12	0.35	0.3^{ns}	0.002	0.05	20.3^3	0.17	0.41
Wf	25.6^3	0.20	−0.45	47.2^3	0.32	−0.57	12.0^3	0.11	−0.33	17.4^3	0.15	−0.39
Al	19.4^3	0.16	0.40	30.7^3	0.24	0.49	3.0^{ns}	0.03	0.17	21.5^3	0.18	0.42
Dm	10.1^2	0.10	0.30	15.0^3	0.13	0.36	0.2^{ns}	0.002	0.04	20.0^3	0.17	0.41
Ic	68.7^3	0.41	0.64	29.5^3	0.23	0.48	0.2^{ns}	0.002	0.04	187.8^3	0.65	0.81
Fhi	48.8^3	0.33	0.57	26.9^3	0.21	0.46	0.04^{ns}	0.0004	0.02	183.5^3	0.65	0.81
Qbr	161.6^3	0.62	0.79	52.0^3	0.34	0.59	3.8^{ns}	0.04	0.19	312.8^3	0.76	0.87
pH	124.4^3	0.56	0.75	44.8^3	0.31	0.56	18.5^3	0.16	0.40	87.2^3	0.47	0.68
Cn	74.1^3	0.43	−0.65	29.7^3	0.23	−0.48	0.9^{ns}	0.008	−0.09	212.8^3	0.68	−0.83
Tu	116.1^3	0.54	−0.73	34.7^3	0.26	−0.51	5.0^1	0.05	−0.22	236.9^3	0.70	−0.84
Su	148.8^3	0.60	−0.77	59.9^3	0.38	−0.61	12.3^3	0.11	−0.33	138.9^3	0.58	−0.76

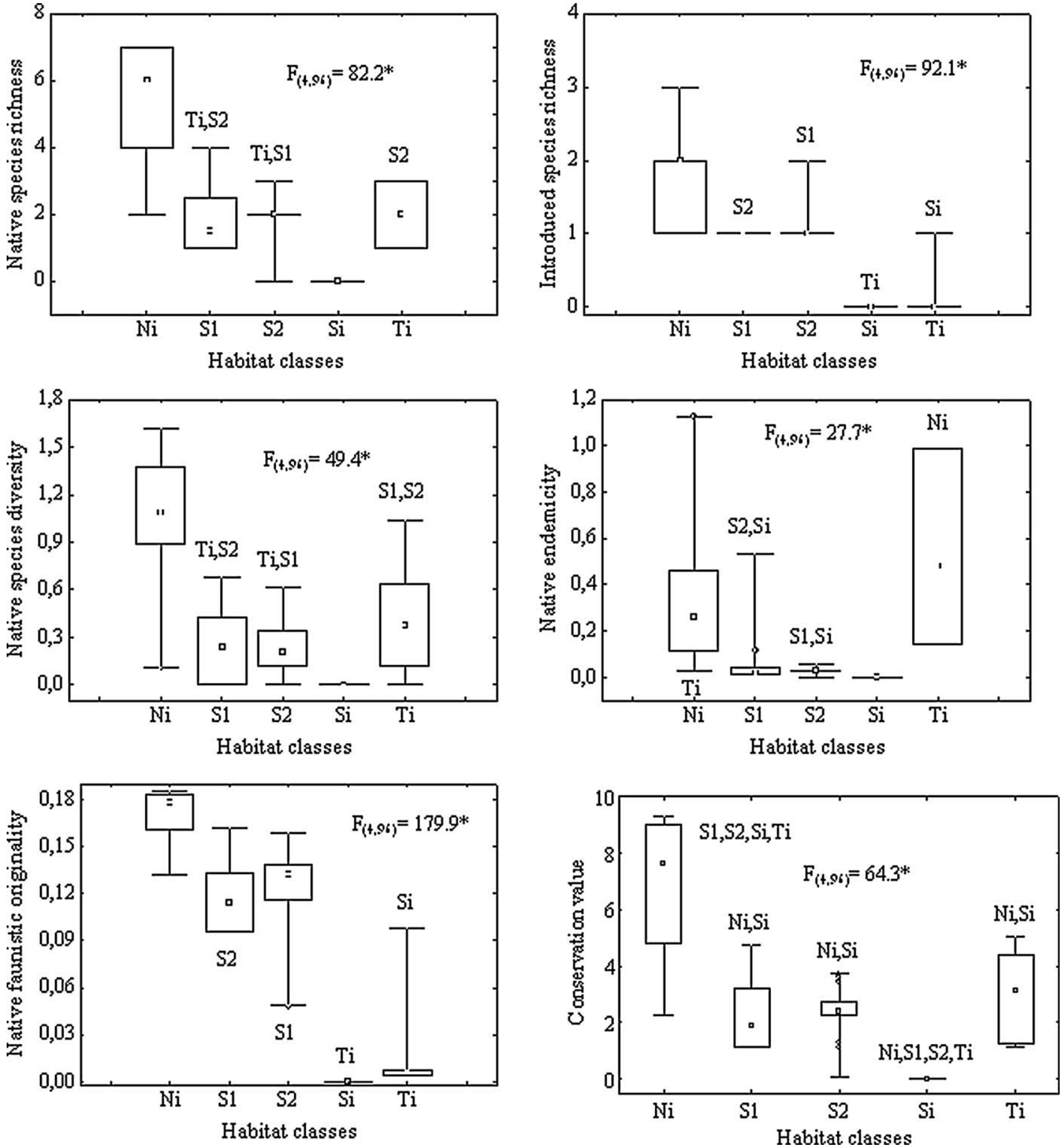

Fig. 5. Differences in the biodiversity indices and conservation value across all habitat classes. Si, severely impacted sites, I1–I3, gradient of sites with varying degrees of impact, Ni, minimally impacted sites (see *Materials and methods*). $^*P < 0.0001$; no significant differences (Tukey's HSD test for paired comparisons) are shown above or below each box. Quadrats, medians, box, percentiles (coefficient = 25), whiskers, the highest and lowest values, excluding outliers, circles, outliers (coefficient = 1.5).

On the other hand, presence–absence data are the basis of some of the most used biotic indices around the world (RIVPACS and AUSRIVAS; Hawkins et al. 2000; Chessman et al. 2008). Therefore, according to its widespread use in biological indices, if the presence–absence data allows a fine approximation of the "health status" of the water bodies, studies of abundance can be, *a priori*, discarded.

Species distribution and sensitivity to AMD-related features

The parameters defining the variables potentially disturbed by humans (see Table 1) are not homogeneously distributed along the environmental gradients. Distribution of freshwater molluscs along these gradients indicates that some species have developed responses to water deficit and to changes in physical and chemical

Table 4
Regression models for the relationships between introduced species richness (Isr) and conservation value (Cv) and environmental variables with PC1 and PC2 loadings >0.60 (see Table 2). R^2: adjusted R^2, β: standardized regression coefficient, 1: $P<0.05$, 2: $P<0.01$, 3: $P<0.001$.

	Isr			Cv		
	$F_{(1,99)}$	R^2	β	$F_{(1,99)}$	R^2	β
At	21.2[2]	0.18	−0.42	26.7[2]	0.20	−0.46
Pr	23.8[2]	0.19	0.44	9.5[1]	0.08	0.29
Wf	20.5[2]	0.17	−0.41	24.8[2]	0.19	−0.45
Al	17.1[2]	0.15	0.38	16.7[2]	0.13	0.38
Dm	20.6[2]	0.17	0.41	7.8[2]	0.06	0.27
Ic	120.32	0.55	0.74	60.1[2]	0.37	0.61
Fhi	133.7[2]	0.57	0.76	41.5[2]	0.30	0.54
Qbr	170.2[2]	0.63	0.79	145.9[2]	0.59	0.77
pH	68.9[2]	0.41	0.64	130.5[2]	0.56	0.75
Cn	156.32	0.61	−0.78	65.6[2]	0.39	−0.63
Tu	158.8[2]	0.61	−0.78	101.7[2]	0.50	−0.71
Su	95.1[2]	0.50	−0.70	141.1[2]	0.58	−0.77

environment, and are distributed according to their intrinsic tolerance levels. Sensitivity-tolerance values of different species can be interpreted according to four models, as defined by the assemblages related to PC1 (Fig. 3):

(1) Tolerant-eurychoric species widely distributed throughout both basins (native *Ancylus fluviatilis* and introduced *Physella acuta*) display an opportunistic behaviour according to their greater adaptability to environmental stressors. Data from this study are consistent with the results reported by other authors in the sense that they are Palaearctic-widely distributed species (Vidal-Abarca and Suárez 1985; Kerney 1999; Glöer 2002) capable of living in highly stressful polluted (Godfrey 1978; Chaisemartin 1981; Cheung and Lam 1998; Flessas et al. 2000; Karouna-Reiner and Sparling 2001; Graça et al. 2004), eutrophicated (Domezain et al. 1987; Camargo et al. 2005; Leitao et al. 2007), dry (Boulton et al. 1992; Bonada et al. 2006; Sheldon and Thoms 2006) or AMD-impacted environments (Gerhardt et al. 2004; Lewin and Smolinski 2006). Therefore, these species appear homogeneously distributed throughout both basins in almost pristine and moderately impacted environments, always avoiding sites with tidal influence.

(2) Sensitive headwater species (native *A. wolfi*, *S. palustris*, *U. delphinus*, *P. casertanum* and *P. personatum*, and introduced *Potamopyrgus antipodarum* and *Gyraulus chinensis*) are adapted to well-preserved environments, with stable water supply and high spatial complexity. The evaluation of these species implies more difficulty in the sense that it is possible that the values of sensitivity-tolerance of different species can be misinterpreted and confounded with their specific habitat preference. On the other hand, available references related to their habitat use are very scarce and in most cases referred to genera or families (Gerhardt 1992; Gallardo et al. 1994; Karouna-Reiner and Sparling 2001; Lewin and Smolinski 2006; Niggebrugge et al. 2007; Petrin et al. 2007). The case of *A. wolfi* is especially problematic because it is a newly described species (Arconada and Ramos 2007; Arconada et al. 2007) and there are no references to their ecology. A more detailed study of these headwater-species would therefore be necessary to redefine the results of this study.

(3) Sensitive mouth species (*H. acuta*, *Peringia ulvae* and *M. myosotis*). Salt-marsh halophilous habitats are more stressful and represent an interface between freshwater habitats and marine conditions. Species occurring there, regardless of their degree of sensitivity to contaminants, develop adaptive mechanisms to minimise the effects of changes in salinity and conductivity according to the tidal rhythms (Blandford and Little 1983; Graham 1988; Hoeksema 1998; Barnes 1999; Bruyndoncx et al. 2002).

(4) Moderately sensitive species (natives *R. balthica*, *G. truncatula*, *Planorbarius metidjensis* and *Planorbis carinatus* and introduced *F. fragilis*). These species develop intermediate adaptive patterns to stressors and show sensitivity to the environmental gradients at different intensities, preferably occupying environments with low natural or anthropogenic impact and avoiding heavily polluted environments (see Fig. 3).

The presence–absence ANOVA analysis identified significant differences in the response of each native species to single gradients of disturbance (see Fig. 4) and provided information that helps to refine the nature of sensitive-tolerant species to AMD stressors. *Ancylus fluviatilis* and *Planorbarius metidjensis*, species previously defined as tolerant and moderately sensitive, respectively, actively select environments with high hydrological stress in relation to low pH and moderately high concentration of sulphides. *A. wolfi*, *G. truncatula*, *R. balthica* and *P. casertanum* appear as the paradigm of the sensitive species, having been found only in unpolluted environments. The low frequency of occurrence of the remaining native species (less than 10 sites) prevents an accurate diagnosis of their sensitivity to stressors.

This study confirms, without any doubt, the tolerant nature of *Physella acuta*, *Ancylus fluviatilis* and, to a lesser extent, *Planorbarius metidjensis* to natural and anthropogenic stressors. Nevertheless, we suggest a conservative analysis of the role of freshwater molluscs as bioindicators of environmental stress, in the sense that it is difficult to distinguish the tolerant nature of eurychorous species from their natural phenotypic plasticity and, on the other hand, it is equally difficult to select the criteria to distinguish sensitive species from others with specific habitat preferences. Although much information is available (e.g. Pynnonen 1990; Gundacker 2000; Bonneris et al. 2005; Campanella et al. 2005), further research and more precise analysis on their physiology and ecology are needed to establish the status of freshwater molluscs as bioindicators of environmental stress.

Native freshwater mollusc biodiversity

The combined effects of a changing environment related to heterogeneity, seasonality and mine discharges on freshwater biodiversity have been amply demonstrated (e.g. Braukmann 2001; Cain et al. 2004; Heino et al. 2007). The results of this study show that freshwater mollusc biodiversity indices are mainly associated with patterns of changing environmental features. Water deficit, heterogeneity (Fhi, Qbr) and chemical features (AMD pollution and conductivity gradients) are the main factors influencing changes in native freshwater mollusc biodiversity. High concentrations of sulphides and elevated turbidity and conductivity, coupled with a low pH, seem to be the main causes of the decline in native freshwater mollusc biodiversity.

The greater water supply, buffer effects from summer droughts, heterogeneity and absence of mine contamination contributes to creating suitable and multifaceted habitat conditions of headwater non-polluted environments. Lowland streams, strongly impacted by summer drought and, to a greater or lesser extent, acid drainage, are subjected to a high variability in flow conditions and thus to a greater or lesser dilution of pollutants. Estuarine communities are partially independent of freshwater supply and are deeply influenced by tidal conditions.

On account of a more stable water supply, scarcity of droughts and high habitat complexity, biodiversity of upper sites are higher than those in middle reaches in which, as the dry season progress, streams are reduced to isolated pools with incremented hypoxia, hyperthermia, eutrophic conditions and contaminant concentrations that increase the stress of biological communities (Gasith and Resh 1999; Bonada et al. 2006; Bonada et al. 2007). The highest biodiversity indices in headwater habitats may be related to three types of requirements: (1) physiological: maximisation of the input of available environmental calcium results in low energetic costs to build their shells (Wäreborn 1970; Wiederholm and Eriksson 1977; Horsák and Hákek 2003); (2) trophic: high pH results in major presence of in-stream vegetation and epilithic algal flora which may allow consumers to invest less energy in searching for food and thus increase their feed efficiency (Lewin and Smolinski 2006; Ortiz and Puig 2007); and (3) structural complexity: high habitat heterogeneity increases the possibility of finding refuge from predators (Turner et al. 1999; Rundle and Brönmark 2001; Dalesman et al. 2007). Similarly, the high degree of biodiversity of mouth assemblages is related to euryhaline species adapted to permanent tide-dependent streams with elevated water conductivity levels (Blandford and Little 1983). In contrast, freshwater mollusc assemblages in unstable middle reaches depend mostly on colonisation/extinction dynamics and biodiversity is regulated mainly by their tolerance to local water pollution and the cyclic increase or decrease in water availability (Øakland 1983; Harvey and McArdle 1986; Winterbourn and McDiffett 1996; Lewin and Smolinski 2006).

Introduced species and conservation

One of the main threats to the integrity of the freshwater mollusc fauna is the introduction of alien species (Prenda et al. 2006; García-Berthou et al. 2007). The presence of introduced species of freshwater molluscs is widespread in the water bodies of the south-western Iberian Peninsula: 16.1% of the mollusc species are introduced, having been found in the 1–100% of the studied watercourses (Pérez-Quintero et al. 2004; Pérez-Quintero 2007; Pérez-Quintero 2009) and in most of the basins in the Iberian Peninsula (Vidal-Abarca and Suárez 1985; Pérez-Quintero 2008; Sousa et al. 2008a; Sousa et al. 2008b).

Many studies have demonstrated the relationship between colonisation by alien species and local extinction events (e.g. Ricciardi and Rasmussen 1999; Burlakova et al. 2000; Hakenkamp et al. 2001; Lydeard et al. 2004; Clavero and García-Berthou 2005; Dudgeon et al. 2006). The results of this study show that the major environmental constraints for the introduced species richness are similar to those observed for native species richness (see Tables 3 and 4), but, nevertheless, introduced species are more sensitive to environmental heterogeneity (Fluvial heterogeneity index, In-stream cover). We can assume, *a priori*, that this strong competition for environmental resources between native and introduced species, joint to other biotic interactions (mainly trophic and reproductives, not addressed in this work) and the impact of human activities, may result, in a not too distant future, in local extinction events of native freshwater mollusc fauna (Strayer et al. 2004; Dudgeon et al. 2006; Régnier et al. 2009).

The statutory protection is highly developed in the study area. Both basins are among the Natural Park "Sierra de Aracena y Picos de Aroche" and the Natural Landscape "Sierra Pelada" (both in the north), the Natural Reserve "Marismas del Odiel" and the Site of Community Importance "Estuario del Río Tinto" (both in the south, bordering the Atlantic Ocean), the Site of Community Importance "Andévalo Occidental" (west) and the Ecological Corridors "Río Guadiamar" and "Rio Tinto", the Sites of Community Importance "Doñana" and "Marismas y Riberas del Río Tinto" and the Protected Landscape "Rio Tinto" (all in the east) (see Fig. 1).

The water courses located in protected environments have unequal conservation value depending on their location: (1) the high species richness and conservation value of sites within the Natural Park "Sierra de Aracena and Picos de Aroche" are those of an environment with reduced water deficit, high spatial heterogeneity and absence of widespread contamination, which allows maintenance of stable populations of stenochoric headwater species; (2) in contrast, the low conservation value of the middle reaches, as well as those located in protected environments, is highly related to the strong seasonality of streams and the greater or lesser AMD impact they support; (3) the conservation value is somewhat higher in tidal environments than in the previous ones due to the lower number of introduced species and the stability of their populations.

Although there are many institutional protection figures in the study area, the territorial protection may not be sufficient to ensure the integrity of watercourses, because water management in Spain is almost independent from the administration of protected areas. So, from a conservationist point of view, plans for the management of freshwater mollusc communities in strongly stressed Mediterranean environments should involve coordination between territorial protection and water management.

In conclusion, restoration programs that integrate physicochemical and biological characteristics of both basins should be the primary targets for future management strategies. From a physicochemical point of view, the Regional Administration should propose programmes to: (1) determine how AMD pollution alters the normal functioning of Mediterranean watercourses and becomes a barrier to dispersal of freshwater biocoenosis; (2) remediate the rivers affected by AMD; (3) propose preventive mechanisms to avoid more degradation of habitats; and (4) connect the degraded river stretches with non-polluted ones by means of fluvial corridors, creating buffer zones between habitats and, consequently, increasing the biodiversity of the zones (Bonn and Gaston 2005; Mancini et al. 2005).

From a biological point of view, it is necessary: (1) to increase the understanding of biological and ecological requirements of Mediterranean freshwater mollusc species, especially those poorly known Iberian species (e.g. *A. wolfi*, *S. palustris*) and (2) the detection and control of the dispersal of exotic species like *Potamopyrgus antipodarum*, *Physella acuta*, *F. fragilis*, *Gyraulus chinensis* or *Corbicula fluminea* (the latter present in the adjacent Guadiana and Guadalquivir River basins, Pérez-Quintero 2007; Pérez-Quintero 2008) to avoid the main biological threat facing native freshwater mollusc species.

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