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# THE INFLUENCE OF WATER QUALITY AND STREAM HABITAT ON WATER BEETLE ASSEMBLAGES IN TWO RIVERS IN NORTHWEST SPAIN

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WATER QUALITY
STREAM HABITAT
ENVIRONMENTAL IMPACT
WATER BEETLE
NORTHWEST SPAIN

ABSTRACT. – The objective of this study was to identify the response of aquatic Coleoptera to water quality and habitat variables in two rivers in northwest Spain with different degrees of contamination. The water beetle assemblages of the Lagares and Miñor Rivers were studied during an annual cycle (May 2001-January 2002). Several physical and chemical variables, in addition to habitat and spatial factors were measured at each site. The environmental variables were then subjected to PCA to facilitate the interpretation of the principal components. The structure of the assemblage was evaluated for different richness measures: Species richness rarefied (ES); Abundance (N) AND Shannon-Wiener Diversity Index (H'). Regression analysis was used to examine the relationships between the richness measures and the water quality variables and the relationship between species and environmental variables was explored by Canonical Correspondence Analysis (CCA). Differences in assemblage structure were examined using analysis of similarities with Bray-Curtis index. We found a total of 35 species of Coleoptera belonging to 10 families, 37 % of which are endemic to the Iberian Peninsula. All richness measures decrease in degraded sites, with a negative correlation between these factors and sites with high levels of contamination. Data analyses also indicated significant differences among the assemblages of non-polluted and polluted waters. CCA showed that most of the species seemed to be sensitive to pollution, only *Limnius volckmari* (Panzer) and *Elmis aenea* (Müller) might be considered tolerant species.

## INTRODUCTION

Rivers are ecosystems of great ecological value, but their special typology makes them fragile and vulnerable to environmental changes, especially those related to disturbances of anthropogenic origin, which often implies irreversible degradation of their biota (Beasley & Kneale 2003, Dahl *et al.* 2004).

The Water Framework Directive (WFD 2000/60/EC) establishes the composition and abundance of benthic fauna, including Coleoptera, as a parameter for calculating the ecological state of a river, and uses the presence/absence of certain taxa to define the state of and classify a watercourse. The presence of certain species in sites, affected or not affected by contamination, turns them into indicators of their environmental quality (Council of the European Communities 2000).

The negative influence of pollution on macroinvertebrate fauna has been described in different works (Elbaz-Poulichet *et al.* 1999, Blasco *et al.* 2000, Smolders *et al.* 2003, Dahl *et al.* 2004, Nummelin *et al.* 2007), but few studies describe the effects of contamination on water beetle fauna. In NW Spain, some studies emphasize the possible negative influence of pollution on aquatic Coleoptera assemblages (García Criado & Fernández Aláez 1995, 2001, García-Criado *et al.* 1999, Felpeto González 1999, Pérez-Bilbao & Garrido 2009).

Beetles represent one of the largest orders of aquatic animals and they are widely distributed in running water systems (Segura *et al.* 2007). In the Iberian Peninsula this group of insects is considerably rich in endemics (Ribera 2000, Garrido & Sáinz-Cantero 2004, Garrido & Munilla 2008, Pérez-Bilbao & Garrido 2008). Aquatic Coleoptera are often used as indicators of biodiversity in freshwater habitats (Foster *et al.* 1990, 1992, Ribera & Foster 1992, Eyre *et al.* 1993, Moreno *et al.* 1997, Sánchez-Fernández *et al.* 2006), and as diagnostic indicators of water quality (Compin & Céréghino 2003, Segura *et al.* 2007). In spite of this, ecological studies of aquatic Coleoptera assemblages in Iberian Peninsula are not common, though at present their number has increased with contributions by Paz (1993), García Criado & Fernández Aláez (1994), Lozano-Quilis *et al.* (2001), Valladares *et al.* (2002) and Fernández-Díaz *et al.* (2008).

The aim of this study is to verify which of the different physical, chemical, and habitat variables are the most important in relation to the distribution of water beetle assemblages. These variables can thus be considered representative when evaluating differences in the faunal composition of rivers located close to each other, but with different degrees of contamination. It will also identify species that are tolerant to pollution, and those that are sensitive to environmental impact.

We specifically addressed species richness-environment and assemblage composition-environment relationships. We expected that species richness measures and assemblage composition of beetles should primarily show responses to habitat structural characteristics and secondarily to stream size (longitudinal gradient) and water chemistry variation. However, given differences between the characteristics of beetles and other invertebrate groups, patterns differing from that expectation might also occur for beetles. We tested the hypotheses that beetle biodiversity would show either (1) variation in stream habitat structure (Segura *et al*. 2007); (2) variation with regard to longitudinal gradient (Miserendino & Archangelsky 2006) or (3) responses to variations in water quality variables (García-Criado *et al*. 1999).

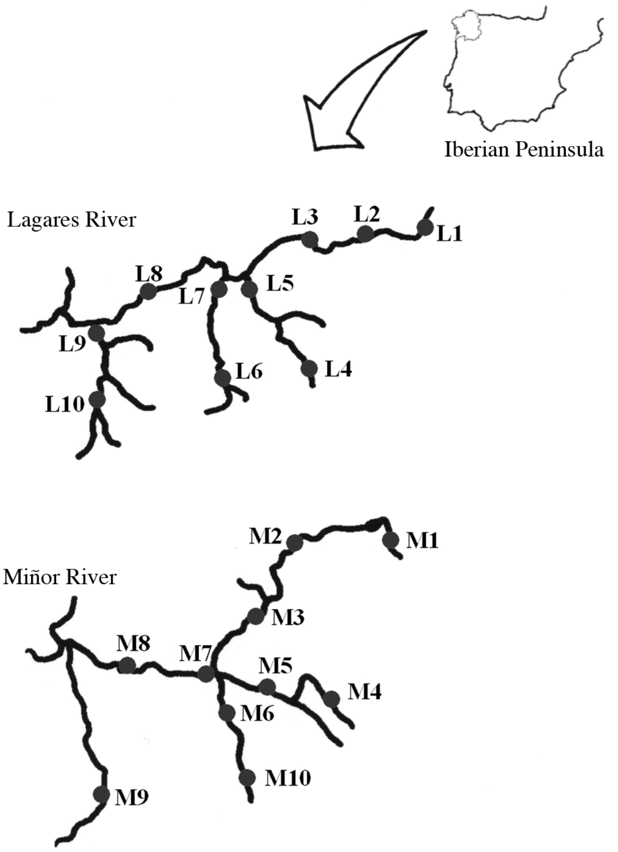

Fig. 1. – Location of the Lagares and Miñor Rivers in northwest Spain.

## MATERIALS AND METHODS

*Study area*: The study was conducted in the Lagares and Miñor Rivers located in the south of Galicia, NW Spain (Fig. 1). The Lagares River runs almost entirely through the urban area of Vigo, a city with approximately 300,000 inhabitants. This river has undergone a profound change in its structure, especially channelling, as a result of the growth of the city and rapid industrial development with the consequent establishment of industries on its banks. The Miñor River runs mostly through rural and semi-urban areas and its course has not been altered very much. Both rivers flow in an EW direction into the Atlantic and their catchment basins are separated by a series of mountains of over 400 metres of altitude. According to WFD (Annex II, System A), both rivers were classified as: Iberian-Macaronesian ecoregion, siliceous, lowland/mid-altitude and small.

Table I. – Sampling sites (L: Lagares; M: Miñor), location, altitude and land uses near the river banks.

| Code | Name | Coordinates | Altitude | Land use |
|---|---|---|---|---|
| L1 | Aeropuerto | 42º13’47”N, 8º38’12”W | 330 | transport system |
| L2 | Rans | 42º13’44”N, 8º39’04”W | 310 | urban, industrial |
| L3 | Sello | 42º13’20”N, 8º40’14”W | 240 | urban, industrial |
| L4 | San Cribán | 42º11’51”N, 8º41’27”W | 220 | agriculture |
| L5 | Sabu | 42º12’47”N, 8º42’14”W | 230 | urban, industrial |
| L6 | Beade | 42º10’53”N, 8º42’48”W | 215 | urban, industrial |
| L7 | Seur | 42º12’45”N, 8º42’44”W | 200 | urban, industrial |
| L8 | San Andrés | 42º12’34”N, 8º44’44”W | 140 | industrial |
| L9 | Rial | 42º12’04”N, 8º45’49”W | 120 | urban, industrial |
| L10 | Fragoselo | 42º10’55”N, 8º45’41”W | 220 | agriculture |
| M1 | Zamans | 42º09’30”N, 8º41’24”W | 395 | woodlands, agriculture |
| M2 | Outeiro | 42º08’52”N, 8º44’07”W | 240 | woodlands, agriculture |
| M3 | Pego Negro | 42º07’35”N, 8º44’47”W | 150 | agriculture |
| M4 | Campo Grande | 42º06’02”N, 8º42’36”W | 380 | woodlands, agriculture |
| M5 | Morgadans | 42º06’51”N, 8º42’06”W | 300 | woodlands, agriculture |
| M6 | Enxertos | 42º06’28”N, 8º45’31”W | 150 | urban |
| M7 | Gondomar | 42º06’37”N, 8º45’56”W | 110 | urban |
| M8 | Covelas | 42º06’46”N, 8º47’57”W | 90 | urban, agriculture |
| M9 | Sequiña | 42º05’59”N, 8º47’01”W | 180 | woodlands, agriculture |
| M10 | Remedios Costa | 42º06’10”N, 8º45’16”W | 325 | woodlands, agriculture |

*Sampling methods and variables measured*: We sampled 20 sites (10 in Lagares River and 10 in Miñor River) for one year (May 2001-January 2002), in four seasons (spring, summer, autumn and winter). The selection of sampling sites was based on land uses near the river banks (woodlands, agriculture, transport system, urban areas, and industrial activities) in conjunction with some habitat factors (Beasley & Kneale 2003) (Table I).

In each site we sampled in all types of substrate for a standardized time (five minutes). The fauna was collected with an entomological water net of 30 cm

Table II. – Abundance of species in Lagares and Miñor Rivers according to its biogeography categories (see Ribera *et al*. 1998).

| Species | Code | Lagares River | Miñor River |
|---|---|---|---|
| Endemics | | | |
| *Nebrioporus carinatus* (Aubé) | *Neb car* | | 1 |
| *Stictotarsus bertrandi* (Legros) | *Sti ber* | | 2 |
| *Hydraena barrosi* d'Orchymont | *Hyd bar* | 2 | 67 |
| *Hydraena brachymera* d'Orchymont | *Hyd bra* | | 53 |
| *Hydraena corinna* d'Orchymont | *Hyd cor* | 5 | 7 |
| *Hydraena iberica* d'Orchymont | *Hyd ibe* | | 84 |
| *Hydraena lusitana* (Berthélémy) | *Hyd lus* | | 19 |
| *Hydraena sharpi* Rey | *Hyd sha* | | 4 |
| *Hydraena unca* Valladares Díez | *Hyd unc* | | 2 |
| *Elmis perezi* Heyden | *Elm per* | 3 | 35 |
| *Limnius perrisi carinatus* (Pérez-Arcas) | *Lim per* | 49 | 134 |
| *Oulimnius bertrandi* Berthélémy | *Oul ber* | | 169 |
| *Oulimnius tuberculatus perizi* (Sharp) | *Oul tub* | 5 | 65 |
| Trans-Iberian | | | |
| *Haliplus lineatocollis* (Marsham) | *Hal lin* | 1 | |
| *Orectochilus villosus* (O. F. Müller) | *Ore vil* | | 17 |
| *Hydroporus tessellatus* (Drapiez) | *Hdp tes* | | 2 |
| *Laccophilus hyalinus* (De Geer) | *Lac hya* | 1 | |
| *Hydrochus angustatus* Germar | *Hdc ang* | | 4 |
| *Anacaena globulus* (Paykull) | *Ana glo* | 1 | 15 |
| *Laccobius sinuatus* Motchulsky | *Lcb sin* | 1 | 1 |
| *Megasternum concinnum* (Marsham) | *Meg con* | 2 | |
| *Esolus parallelepipedus* (Müller) | *Eso par* | | 178 |
| *Limnius opacus* Müller | *Lim opa* | | 5 |
| *Dryops luridus* (Erichson) | *Dry lur* | 1 | 1 |
| Northern | | | |
| *Hydroporus nigrita* (Fabricius) | *Hdp nig* | | 1 |
| *Helophorus flavipes* Fabricius | *Hel fla* | | 1 |
| *Dupophilus brevis* Mulsant & Rey | *Dup bre* | 1 | 11 |
| *Elmis aenea* (Müller) | *Elm aen* | 38 | 18 |
| *Elmis maugetii maugetii* Latreille | *Elm mau* | 2 | 102 |
| *Elmis rioloides* (Kuwert) | *Elm rio* | | 68 |
| *Limnius volckmari* (Panzer) | *Lim vol* | 219 | 104 |
| *Oulimnius troglodytes* (Gyllenhal) | *Oul tro* | 2 | |
| Non-assigned (larvae) | | | |
| *Agabus* sp. | *Aga* sp | 3 | 1 |
| *Elodes* sp. | *Elo* sp | | 5 |
| *Hydrocyphon* sp. | *Hcy* sp | | 85 |

diameter, 60 cm depth and 0.5 mm mesh. The specimens were stored in a 4 % formaldehyde solution and taken to the laboratory, where they were sorted out and identified. After being studied, they were conserved in 70° alcohol and deposited in the scientific collection of the Entomology Laboratory at Vigo University.

The following water quality variables were measured at each site: temperature, dissolved oxygen, pH, electrical conductivity, total dissolved solids (TDS), total suspended solids (TSS), suspended particulate matter (SPM), calcium, iron, potassium, magnesium, sodium, nitrate, nitrite, silicate, chloride, aluminium, boron, cadmium, chrome, copper, lithium, manganese, nickel, lead, zinc, ammonium, phosphorus, sulphate, fluoride and mercury. Water quality factors were measured following APHA (1998) methods. Additionally, the following habitat characteristics (physical factors and spatial variables) were measured: site altitude, distance from source and sea, depth, width, stream velocity and dominant substrate (mud, stone or vegetation). These characteristics are related to water beetle distribution, and are very important for establishing ecological river

Table III. – Physical, chemical and habitat variables of the 20 sites sampled in Lagares and Miñor Rivers in four seasonal campaigns (from May 2001 to January 2002).

| Variable | Mean ± standard deviation | Minimum | Maximum |
|---|---|---|---|
| Altitude m | 227 ± 87 | 90 | 395 |
| Distance of Source m | 5425 ± 4646 | 350 | 14400 |
| Distance of Sea m | 6820 ± 3886 | 1500 | 15150 |
| Stream Width m | 3.21 ± 1.53 | 1.22 | 7.1 |
| Stream Depth cm | 24.88 ± 12.06 | 6.8 | 63.5 |
| Stream Velocity Km/h | 2.5 ± 0.71 | 0.68 | 4.8 |
| T° air °C | 17.78 ± 4.44 | 8 | 29 |
| T° water °C | 14.90 ± 2.38 | 9.7 | 20.9 |
| pH | 6.59 ± 0.63 | 5.02 | 9.32 |
| Conductivity mS cm$^{-1}$ | 86.24 ± 50.66 | 21 | 193 |
| Dissolved oxygen % | 94.86 ± 9.43 | 45 | 113 |
| Dissolved oxygen mg/l$^{-1}$ | 9.45 ± 1.04 | 3.9 | 11.3 |
| TDS mg/l$^{-1}$ | 37.81 ± 22.31 | 10 | 121 |
| TSS mg/l$^{-1}$ | 39.79 ± 112.51 | 0.11 | 658 |
| SPM mg/l$^{-1}$ | 5.72 ± 11.77 | 0.01 | 66 |
| Ca mg/l$^{-1}$ | 3.47 ± 3.19 | 0.24 | 12.81 |
| Fe mg/l$^{-1}$ | 0.02 ± 0.03 | 0.01 | 0.22 |
| K mg/l$^{-1}$ | 1.26 ± 0.89 | 0.05 | 4.10 |
| Mg mg/l$^{-1}$ | 1.67 ± 1.21 | 0.33 | 5.13 |
| Na mg/l$^{-1}$ | 8.71 ± 4.68 | 3.27 | 20.87 |
| $NO_{3-}$ mg/l$^{-1}$ | 1.45 ± 1.22 | 0.12 | 5.18 |
| $SiO_2$ mg/l$^{-1}$ | 3.77 ± 2.36 | 0.18 | 8.08 |
| $Cl_-$ mg/l$^{-1}$ | 11.84 ± 5.40 | 4.95 | 26.77 |
| Al mg/l$^{-1}$ | 0.05 ± 0.15 | < 0.002 | 1.15 |
| B mg/l$^{-1}$ | 0.02 ± 0.02 | < 0.0015 | 0.08 |
| Cd mg/l$^{-1}$ | < 0.001 ± < 0.001 | < 0.001 | < 0.001 |
| Cr mg/l$^{-1}$ | < 0.004 ± < 0.004 | < 0.004 | < 0.004 |
| Cu mg/l$^{-1}$ | 0.01 ± 0.02 | 0.001 | 0.13 |
| Li mg/l$^{-1}$ | 0.0013 ± 0.0004 | 0.001 | 0.002 |
| Mn mg/l$^{-1}$ | 0.017 ± 0.022 | 0.001 | 0.14 |
| Ni mg/l$^{-1}$ | 0.007 ± 0.0003 | < 0.005 | 0.008 |
| Pb mg/l$^{-1}$ | < 0.001 ± < 0.001 | < 0.001 | < 0.001 |
| Zn mg/l$^{-1}$ | 0.005 ± 0.004 | < 0.001 | 0.025 |
| $NH_4$ mg/l$^{-1}$ | 0.06 ± 0.06 | 0.005 | 0.29 |
| $NO_{2-}$ mg/l$^{-1}$ | 0.014 ± 0.011 | < 0.005 | 0.06 |
| $PO_{4\,3-}$ mg/l$^{-1}$ | 0.07 ± 0.14 | < 0.005 | 0.6 |
| $SO_{4\,2-}$ mg/l$^{-1}$ | 10.41 ± 4.20 | < 5 | 19.64 |
| $F_-$ mg/l$^{-1}$ | < 0.1 ± < 0.1 | < 0.1 | < 0.1 |
| Hg mg/l$^{-1}$ | < 0.1 ± < 0.1 | < 0.1 | < 0.1 |

structure (Dahl *et al*. 2004, Tomanova *et al*. 2006, Baptista *et al*. 2007, Bonada *et al*. 2007, Haidekker & Hering 2008, Heino & Paasivirta 2008) and considered fundamental in the typology of rivers by the WFD.

*Data analysis*: The structure of the assemblage was evaluated for different richness measures: Rarefied species richness (ES); Abundance (N) and the Shannon-Wiener Diversity Index (H'). These indices were selected because they potentially portray important characteristics of assemblages. Analysis of variance (two-way ANOVA) was used to test for significant differences between the two rivers and seasons in both richness measures. ANOVA was run using SPSS version 14.

The environmental variables were then subjected to PCA to facilitate the interpretation of the principal components. Variables with very low values (many of which below the level of detection) were removed. Previously the normality of environmental variables was tested using Kolmogorov-Smirnov and Shapiro-Wilk tests. Also, appropriate transformations (logarithmic) were performed when needed. Separate PCAs were run for physical habitat factors and water chemistry variables to obtain uncorrelated principal components based on each group of variables. Thus, all six principal components generated through the two separate PCAs can be used in regression and canonical correspondence analysis (CCA) without much collinearity among the explanatory variables. PCAs were run with Statistica version 7.

Regression analysis with forward stepwise selection ($\alpha = 0.05$) was used to examine the relationships between the richness measures and the environmental variables (i.e., the six principal components) and spatial variables (i.e., altitude and distance of source and sea). Analyses were run using SPSS version 14.

The influence of environmental variables on beetle species was explored by Canonical Correspondence Analysis (CCA). Species with an abundance of less than 10 specimens were not included in the analysis. For this analysis the used variables were the six principal components (CPC and PPC) and the spatial variables (altitude and distance of source and sea). The statistical significance of each variable selected was judged by a Monte Carlo permutation test. The Canoco for Windows package, version 4.5 (ter Braak & Šmilauer 2002), was used for this multivariate analysis. Finally, we used complete linkage cluster analysis with Bray-Curtis coefficient to cluster the sites into groups. Cluster analysis was run with PRIMER version 6.0

## RESULTS

1,597 specimens belonging to 35 species of 10 families were studied. Of that total, 37 % were species endemic to the Iberian Peninsula, especially the representatives of the Hydraenidae family, since all the present species are Iberian endemics. The other species belong to Trans-Iberian and Northern categories (see Ribera *et al.* 1998) (Table II).

Table III gives the mean, maximum and minimum values of the water quality and habitat variables measured in both rivers during the annual cycle. The sites located in areas with urban and industrial land uses (see Table I) had the highest values of water quality variables.

Principal component analysis on both physical and chemical variables yielded three easily interpretable composite environmental gradients. For physical habitat variables, the first principal component (PPC1) described stream size gradient, with width and depth having positive loadings and vegetation dominant substrate a high negative one (Table IV). Thus, sites with high scores for this component were located next to the mouth, while those with low scores were situated next to the source. The second physical component (PPC2) described longitudinal gradient related with current velocity, with high positive loadings for this variable and stone dominant substrate (Table IV). The third physical component (PPC3) described habitat structure, with high positive loadings for mud dominant substrate (Table IV). The first water chemistry component (CPC1) was primarily a gradient describing water quality, with high positive loadings for Ca, K, Mg, Na, $NO_3$, Cl and conductivity (Table V). Thus, sites with high scores for this component had high values for these variables, while those with low scores had low values for these chemistry variables. The second water

Table IV. – Summary of Principal Components Analysis on the physical habitat variables (PPC). Variable loadings > 0.5 are in bold.

| | PPC1 | PPC2 | PPC3 |
|---|---|---|---|
| Eigenvalue | 2.191 | 1.310 | 0.777 |
| Variance explained % | 36.515 | 21.836 | 12.942 |
| Variable loading | | | |
| Mud | -0.41 | -0.092 | **0.687** |
| Stones | -0.482 | **0.594** | -0.176 |
| Vegetation | **-0.609** | 0.451 | -0.287 |
| Stream Depht | **0.763** | 0.165 | 0.151 |
| Stream Width | **0.696** | 0.373 | -0.091 |
| Stream Velocity | 0.231 | **0.760** | -0.401 |

Table V. – Summary of Principal Components Analysis on the water chemistry variables (CPC). Variable loadings > 0.5 are in bold.

| | CPC1 | CPC2 | CPC3 |
|---|---|---|---|
| Eigenvalue | 7.73 | 2.934 | 2.100 |
| Variance explained % | 32.21 | 12.226 | 8.750 |
| Variable loading | | | |
| Ca | **0.95** | -0.078 | 0.034 |
| Fe | 0.49 | 0.222 | -0.439 |
| K | **0.86** | 0.102 | -0.043 |
| Mg | **0.96** | -0.045 | 0.176 |
| Na | **0.89** | -0.133 | 0.302 |
| $NO_3$ | **0.73** | -0.198 | 0.096 |
| $SiO_2$ | 0.49 | 0.224 | 0.316 |
| Cl | **0.82** | -0.143 | 0.375 |
| pH | 0.27 | 0.253 | 0.437 |
| O2 | -0.11 | **0.795** | -0.026 |
| Conductivity | **0.69** | 0.191 | 0.251 |
| TDS | 0.46 | 0.202 | 0.222 |
| TSS | 0.40 | -0.194 | **-0.650** |
| SPM | 0.43 | -0.165 | **-0.646** |
| Al | -0.04 | 0.195 | 0.160 |
| B | -0.28 | -0.217 | 0.256 |
| Cu | -0.22 | -0.066 | -0.189 |
| Li | -0.01 | 0.005 | -0.234 |
| Mn | 0.45 | -0.117 | -0.070 |
| Zn | -0.11 | -0.273 | 0.220 |
| $NH_4$ | 0.45 | 0.007 | 0.142 |
| $NO_2$ | -0.12 | -0.357 | 0.003 |
| $PO_4$ | -0.33 | **-0.565** | 0.146 |
| $SO_4$ | -0.30 | 0.266 | 0.235 |

Table VI. – Mean, SD and ranges of richness measures of the samples and ANOVA with rivers (1) and season (2) as factors.

| Richness measures | Mean ± SD | Minimum | Maximum | ANOVA Factor 1 | | ANOVA Factor 2 | |
|---|---|---|---|---|---|---|---|
| | | | | F | p | F | p |
| Rarefied richness (SE) | 3.09 ± 2.83 | 0 | 12.61 | 44.267 | < 0.001 | 5.561 | 0.002 |
| Abundance (N) | 19.96 ± 5.07 | 0 | 241 | 8.913 | 0.004 | 4.32 | 0.004 |
| Diversity (H') | 1.12 ± 0.17 | 0 | 2.17 | 50.832 | < 0.001 | 4.827 | 0.004 |

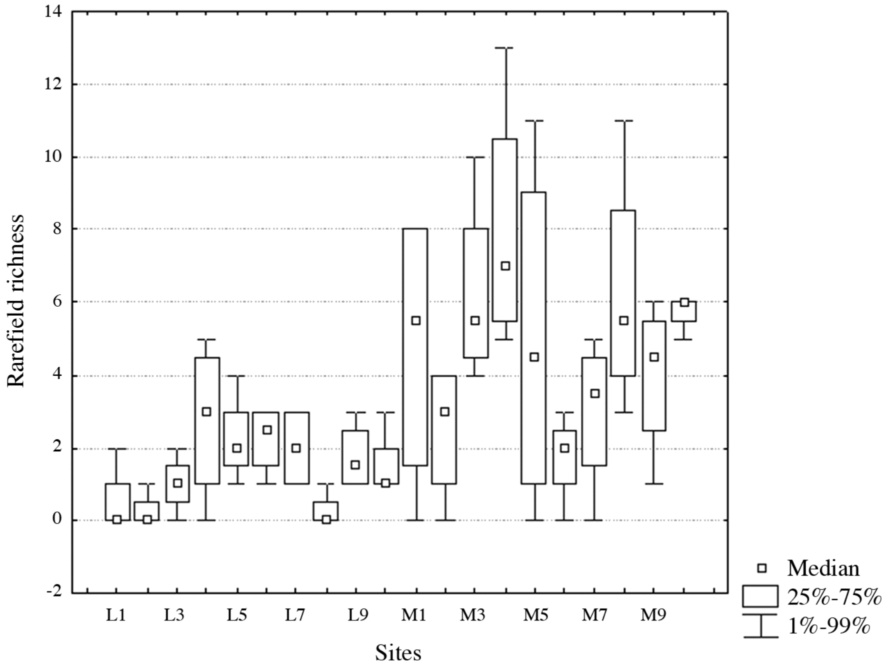

Fig. 2. – Richness (total number of species) in the total samples of the Lagares (L) and Miñor (M) Rivers.

chemistry component (CPC2) was primarily a gradient describing oxygen concentration, with positive loadings for this variable and negative loadings for $PO_4$ (Table V). The third chemical component (CPC3) was primarily a gradient describing suspended solids, with positive loadings for TSS and SPM (Table V).

Richness measures showed considerable variability among sites and seasons as evidenced by the ANOVA (Table VI), with significant differences between the two rivers and seasons in both richness measures. Final regression models revealed a significant negative correlation ($p = < 0.05$) between most chemical variables (i.e., chemistry principal components) and richness measures (Table VII). All richness measures values (ES, N, H') decrease in sites and samples with high values for chemical factors. Also, average values of rarefied richness (ES) are markedly higher in the Miñor than in the Lagares River as shown in Fig. 2.

Figs. 3 and 4 show the results of CCA analysis. The eigenvalues for axes 1-4 were 0.595, 0.452, 0.179 and 0.132. Correlations for axes III and IV with environmental variables were low ($r < 0.5$), and only axes I and II were used for data interpretation. The cumulative percentage of variance for the species-environmental relation was 64.2 for these two axes. The first two canonical axes were significant, as shown by the Monte Carlo permutation test ($p = 0.002$). The overall Monte Carlo test also gave a significance of $p = 0.002$.

The first principal gradient coincides with axis I and is positively correlated with CPC1 ($r = 0.714$), and negatively correlated with PPC2 ($r = -0.5268$). This component describes water quality and is correlated with high values of several chemistry variables. The variations in the values of these variables, which are clear indicators of contamination, seem to be the main factors affecting the distribution of Coleoptera in these rivers. All Lagares River sites are located at the positive end of the axis, and all Miñor River sites are located at the negative end of it (Fig. 3). The majority of species are located on the negative side of this axis (Fig. 4).

The second gradient coincides with axis II and is positively correlated with altitude ($r = 0.868$) and CPC2 ($r = 0.646$) and negatively correlated with distance of source ($r = -0.882$) and PPC1 ($r = -0.668$). CCA established two groups of species of aquatic beetle along the longitudinal gradient (source-mouth) one next to the source and the other next to the mouth.

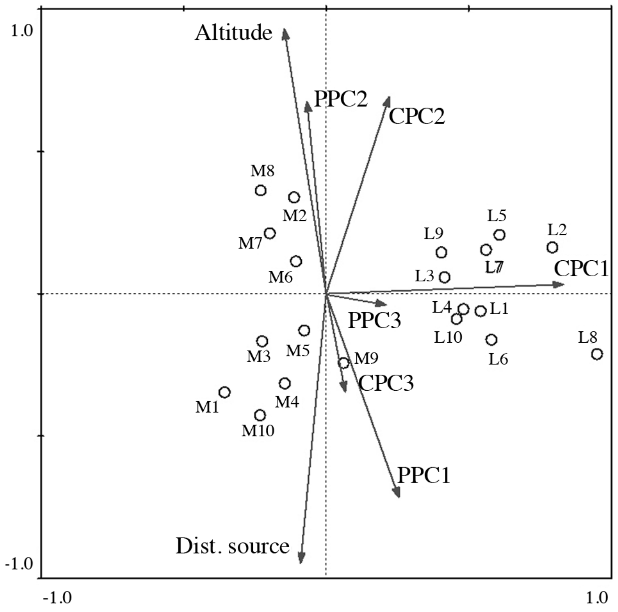

Fig. 3. – Axis 1 and 2 of the Canonical Correspondence Analysis (CCA). Sites and environment biplot.

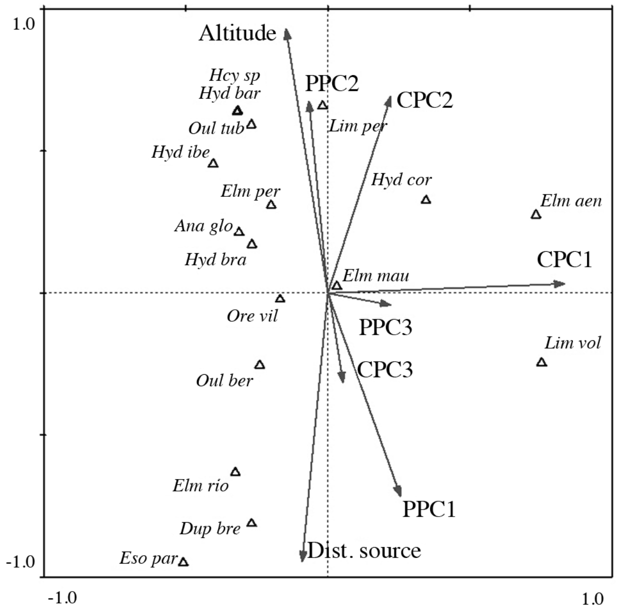

Fig. 4. – Axis 1 and 2 of the Canonical Correspondence Analysis (CCA). Species and environment biplot. (For species names see Table II).

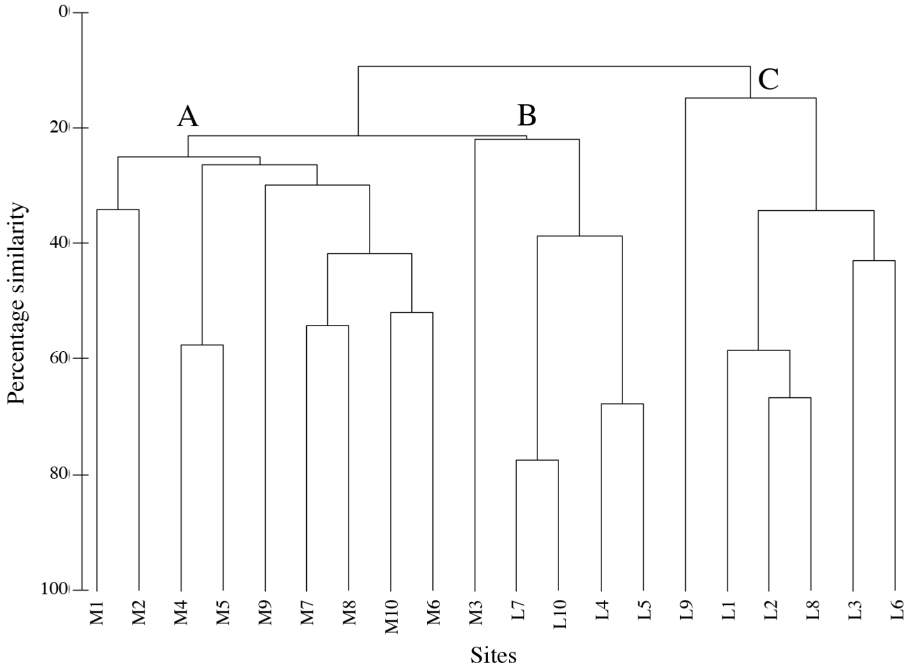

Fig. 5. – Dendrogram from complete linkage clustering based on the Bray-Curtis coefficient. A, B and C refer to the cluster groups.

Cluster analysis provided alternative insights into the similarity of sites with regard to beetle assemblage composition. Figure 5 shows the formation of three clearly separated groups with very low faunal affinity between them (14-22 %). In general, sites from each river were relatively closely situated in the dendrogram. Thus, most Miñor sites were incorporated in the first group (A) and most Lagares sites were incorporated in the third one (C). The second group (B) comprised four sites from the Lagares River and one from the Miñor River.

## DISCUSSION

According to Beasley & Kneale (2003), increasing urbanization and industrialization generates different non-point sources of contamination, causing impairment of water quality of rivers. This environmental impact can be seen in the city of Vigo and its surroundings. The high anthropogenic pressure on the aquatic ecosystems in this region is a consequence of the ever-increasing population and establishment of industries, especially in the banks

Table VII. – Final regression model for the species richness measures. Environmental characteristics were portrayed by PCA from physical (PPC) and chemical (CPC) data.

| Model | b | SE | t | p | R2 |
|---|---|---|---|---|---|
| Rarefied richness | | | | | |
| Constant | 3.088 | 0.256 | 12.076 | < 0.001 | **0.435** |
| CPC1 | -0.449 | 0.094 | -4.793 | < 0.001 | |
| CPC3 | -0.455 | 0.158 | -2.884 | 0.005 | |
| PPC1 | 0.547 | 0.194 | 2.821 | 0.006 | |
| Abundance | | | | | |
| Constant | 19.963 | 3.675 | 5.432 | < 0.001 | **0.286** |
| CPC1 | -3.129 | 1.346 | -2.324 | 0.023 | |
| CPC3 | -6.222 | 2.266 | -2.746 | 0.008 | |
| PPC1 | 7.239 | 2.785 | 2.599 | 0.011 | |
| PPC3 | -7.810 | 3.326 | -2.348 | 0.022 | |
| Diversity | | | | | |
| Constant | 0.664 | 0.057 | 11.590 | < 0.001 | **0.438** |
| CPC1 | -0.106 | 0.021 | -5.037 | < 0.001 | |
| CPC2 | 0.112 | 0.044 | 2.578 | 0.012 | |
| CPC3 | -0.096 | 0.035 | -2.705 | 0.008 | |
| PPC1 | 0.103 | 0.043 | 2.378 | 0.020 | |

of rivers. If we compare the values of chemical variables with the degree of anthropogenic activity on the banks, we can conclude that the sites with moderate or strong activity (industrial land uses) correspond to those whose these variables have higher values.

Different studies have often documented a decrease in species richness, total abundance and diversity in water bodies with high values for chemical variables (Prenda & Gallardo-Mayenco 1996, Heino 2000). In our study, we found a negative correlation between most chemical variables and richness parameters with an important decrease in rarefied richness, abundance and diversity in impacted sites, especially in the Lagares River. The differences between the two rivers in both richness measure values were evident, as we can see from the results of the ANOVA. In the Lagares River, richness values are considerably lower in comparison with the Miñor River and other rivers in northern Spain (Paz 1993, García Criado 1999, Fernández Díaz 2003, Pérez-Bilbao & Garrido 2009). The ANOVA also revealed significant differences between seasons, which shows that sampling in only one season is not enough to evaluate the state of conservation of a habitat, which has already been shown by other papers (García-Criado & Fernández Aláez 2001; Pérez-Bilbao & Garrido 2009).

Multivariate techniques constitute an additional and very useful approach since they provide information on the interaction of a set of environmental variables and their relationship with assemblage distribution (García-Criado *et al.* 1999). In our work the CCA showed two principal gradients, one correlated with contamination of the water and the other correlated with the longitudinal gradient of the rivers. Axis I indicated significant differences between the Lagares and Miñor Rivers. The Lagares River sites are grouped along the positive end of axis (high values for water quality factors), whereas the Miñor River sites are grouped along the negative end of axis (low values for water quality factors). Positively correlated with the "contamination" gradient (CPC1) we found the variables calcium, potassium, sodium, nitrate, magnesium, chloride and conductivity. Dahl *et al.* (2004) and Ortiz *et al.* (2005) also related that high values of nitrate, potassium and magnesium are good indicators of contamination in rivers, coinciding with the results of our study.

The response of beetles to water pollution seemed to define, at least, the species typical of non-contaminated sites. In this sense, ordination analysis identified a group of sensitive species (for example, *H. barrosi, H. brachymera* d'Orchymont and *H. iberica* d'Orchymont), especially evident for those most abundant, whose numbers fall considerably in impacted sites. According to CCA, there is also a group of tolerant species (*Limnius volckmari* (Panzer) and *Elmis aenea* (Müller)), usual to sites affected by pollution; however, they are not really species "characteristic" of polluted sites, since they were also collected at non-impacted sites. These results are in agreement with those found in other studies about Coleoptera (García-Criado *et al.* 1999, García-Criado & Fernández-Aláez 2001, Beasley & Kneale 2003, Nummelin *et al.* 2007, Fernández-Díaz *et al.* 2008).

The degree of tolerance to environmental impacts is variable depending on the taxa. For instance, different species of Hydraenidae have been regarded as non-tolerant to contamination (Bargos *et al.* 1990, Díaz Pazos 1991, Cuppen 1993, García Criado & Fernández-Aláez 1995, 2001, Fernández Díaz *et al.* 2008). As shown by the projection of the CCA axes, most of the Hydraenidae species were sensitive to contamination and were found in sites little affected by pollution. Another group that provides relevant information are elmids. In our study, most elmid species were sensitive to pollution and were associated with little altered sites, on the negative side of the contamination gradient. These results are corroborated by different works that have documented pollution intolerance in different species of Elmidae (Armitage 1980, Puig 1983, Thomas 1988, Richoux & Forestier 1989, Grasser 1994, García-Criado *et al.* 1999, García Criado & Fernández-Aláez 2001, Fernández-Díaz *et al.* 2008)

In this study, only *E. aenea* and *L. volckmari* were tolerant to increasing values for the pollution indicator variables. There are few works that have studied pollution tolerance in *E. aenea*. One of these is Gower *et al.* (1995) who find this species to be non-tolerant to high levels of contamination by heavy metals. According to Heino (2005) it is a "generalist" species, with high tolerance values and occurring across widely varying environmental conditions. The other tolerant species, *L. volckmari*, was the most abundant, frequent and widely-distributed species in the Lagares and Miñor Rivers. This beetle was often the only species present in many sites, occupying the niche left vacant by sensitive species. Literature corroborates our data and indicates the high tolerance of this species to ecological changes. Rico (1992) emphasizes its wide ecological amplitude. Fernández Díaz (2003) associates it with a gradient of mineralization and high conductivity values, while García Criado & Fernández-Aláez (2001) associate it with moderate contamination. However, Gower *et al.* (1995) found this species to be non-tolerant to high levels of contamination by heavy metals and Heino (2005) considered it to be "specialist", occurring only across a limited range of conditions.

CCA axis 2 showed a "longitudinal" gradient of the rivers (source-mouth). In our study we found both positive and negative correlations with this axis. The species form two clear groups: one correlated with sites away from the source and with greater depth and width (PPC1) (e.g., *Esolus parallelepipedus* (Müller)*, Elmis rioloides* (Kuwert) and *Dupophilus brevis* Mulsant & Rey), and the other correlated with sites at a higher altitude, with increased current velocity and high values of dissolved oxygen (e.g., *Limnius perrisi carinatus* (Pérez-Arcas), *Oulimnius tuberculatus perizi* (Sharp) and *Hydraena barrosi* (d'Orchymont). In this sense, longitudinal gradient is a significant discriminating factor for these species. In the Lagares and Miñor Rivers it is the most important factor after the "contamination" gradient (axis 1). Baptista *et al.* (2001), González *et al.* (2003), Heino *et al.* (2005), Hughes (2006) and Leunda *et al.* (2009) have stressed the importance of this factor for different macroinvertebrates and suggested that the faunal composition gradually changed along the longitudinal gradient of rivers. Miserendino & Archangelsky (2006) studied the longitudinal distribution of Coleoptera in the Chubut River (Argentina) and suggested that the change in faunal composition along the river was mainly due to changes in the substrate. This was also observed in our work with respect to dissolved oxygen and current velocity, correlated with the higher altitude and source of rivers.

As expected, most of the sites from each river harboured similar beetle assemblages. The cluster groupings were highly significant, despite the fact that each group (A, B and C) had sites located in different stretches of the rivers and also sites with different habitat structure. Thus, not only the longitudinal gradient, but also localized environmental variables may produce significantly different types of beetle assemblages. In this sense the significant differences between the two rivers in terms of water quality factors is remarkable as was also demonstrated by CCA. The response of beetles to increased pollution seems to define, at least, a community typical of non-polluted areas. It includes species present in sites from the Miñor River (group A). The Lagares sampling sites were grouped into two distinct cluster groups (B and C) indicating that the existence of an assemblage typical of polluted areas was not so clear. Group B was closer to group A than to group C. It is difficult to define the factor grouping these sites (group B) since they have different habitat structure. It could be the altitude (similar in all) or agriculture (principal land use in most of them). However, water quality is probably the factor that separates groups A and B, with a low faunal affinity between them (22 %).

In conclusion, the negative influence of anthropogenic impact, especially water contamination, on aquatic Coleoptera assemblages in the Lagares and Miñor Rivers is evident, as shown by the decrease in the richness attributes in impacted sites. As anticipated, there is a loss of species as land use changes from rural to urban. The data also indicates that the water quality in the Miñor River (mainly rural land uses) is better than in the Lagares River (urban land uses). The Miñor River is less impacted and its waters are less contaminated, although the low values for richness parameters obtained in certain sampling sites indicates an increase in anthropogenic pressure, threatening the conservation of this fauna.

We also have demonstrated that water beetles can be used as indicators of environmental impacts in rivers. Their responses to impacts in rivers differ; the majority of species are not tolerant to increasing contamination and changes in river structure, but *L. volckmari* and *E. aenea* seem to have adapted to these changes and become dominant in the highly disturbed sites. As expected, rare and endemic species appears to be unmistakably associated with good water quality, which highlights the importance of conserving the freshwater habitats. The preservation of species is one of the main factors that should be taken into consideration in developing monitoring systems and assessing reference conditions, as stipulated by the WFD (Council of the European Communities 2000).

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