Departamento de Ecología e Hidrología
Universidad de Murcia

Ecología de las Comunidades de Macroinvertebrados de la Cuenca del Río Segura (SE de España)

Factores ambientales, variabilidad espacio-temporal, táxones indicadores, patrones de diversidad, rasgos biológicos-ecológicos y aplicaciones para la evaluación biológica

The Ecology of Stream Macroinvertebrate Assemblages from the Segura River Basin (SE Spain)

Environmental factors, spatio-temporal variability, indicator taxa, diversity trends, biological-ecological traits and applications for bioassessment

Andrés Mellado Díaz

2005

UNIVERSIDAD
DE MURCIA

DEPARTAMENTO DE ECOLOGÍA
E HIDROLOGÍA

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CERTIFICAN

Que la Memoria que lleva por título "**Ecología de las comunidades de macroinvertebrados de la Cuenca del Río Segura. Factores ambientales, variabilidad espacio-temporal, taxones indicadores, tendencias en diversidad, rasgos biológicos-ecológicos y aplicaciones para la evaluación biológica**" (The Ecology of Stream Macroinvertebrate Assemblages from the Segura River Basin. Environmental factors, spatio-temporal variability, indicator taxa, diversity trends, biological-ecological traits and applications for bioassessment) que presenta el Licenciado D. **Andrés Mellado Díaz**, para la obtención del título de Doctor, ha sido realizada bajo nuestra dirección

Murcia, 16 de Junio de 2005

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CERTIFICA

Que la Memoria que lleva por título "**Ecología de las comunidades de macroinvertebrados de la Cuenca del Río Segura. Factores ambientales, variabilidad espacio-temporal, taxones indicadores, tendencias en diversidad, rasgos biológicos-ecológicos y aplicaciones para la evaluación biológica**" (The Ecology of Stream Macroinvertebrate Assemblages from the Segura River Basin. Environmental factors, spatio-temporal variability, indicator taxa, diversity trends, biological-ecological traits and applications for bioassessment) que presenta el Licenciado D. **Andrés Mellado Díaz**, para la obtención del título de Doctor, ha sido realizada íntegramente en este Departamento, el cuál ha autorizado su presentación ante la Comisión de Doctorado, bajo mi dirección y la de la profesora Dra **Mª Luisa Suárez Alonso**

Murcia, 16 de Junio de 2005

Fdo.: Mª Rosario Vidal-Abarca Gutiérrez

Departamento de Ecología e Hidrología

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Memoria presentada para optar al grado de Doctor

Murcia, Julio 2005

A mis abuelos, mis padres,
mi hermano, mi cuñada, mi sobrino chico, mis amigos,
a Marta

Agradecimientos

Hará unos siete años ya que empecé estas correrías. Quería empezar a hacer memoria, desde el principio, desde la fuente, como el río - valga este símil como homenaje - para hacer una especie de diario "de deriva"; agradeciendo gestos, favores, influjos varios. Tuve suerte de encontrarme con la gente que me encontré. Algunos y algunas ya forman parte de mi de una u otra forma, de mi pasado algunos, pocos, de mi presente la mayoría, que somos jóvenes, y de mi futuro, bueno, eso ya está lejos. Agradeceré sinceramente a todas las personas que me han echado un cable en algún que otro momento, o muchos cables. En siete años da tiempo a casi todo.

Primero recordar a la gente que me metió en este "fregao", a Pepa Velasco y Andrés Millán. A ambos, gracias por todo lo que me aportaron, y me siguen aportando, científica y personalmente. Y a sus crías por ser tan guapas.

A mis Directoras de tesis, Maria Luisa Suárez y Chary Vidal-Abarca, quiero agradecerles todo lo que hemos pasado juntos, muchos raticos buenos de río en río, de estación en estación…y de bar en bar, que en todos los trabajos se fuma. Las discusiones, que siempre son buenas, vuestras ideas propias, y ahora que parece que doy por fin este paso, este último empujoncito (yo es que sin empujones nunca termino las cosas). Por haber confiado todos estos años en este proyecto y haberme dejado hacer y deshacer. Y tantas otras cosas que a veces se agradecen a las buenas directoras. Va por vosotras.

A mis compañeros de trabajo, Jose Luis y Alberto, naturalistas donde los haya, que me metieron el gusanillo de la historia natural, de querer saber mirar. Y por todo el trabajo de campo, y de laboratorio. También pasamos buenas jornadas de risas y cañas, que todo hay que

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Introducción general

Introducción general

Existe un acuerdo generalizado sobre el valor de los invertebrados acuáticos como indicadores de la calidad del agua de ríos, arroyos y casi cualquier ecosistema acuático continental (e.g. Chessman and McEvoy, 1998; Reynoldson, et al., 2001. Metzeling, et al., 2003.). Los estudios sobre su biología y ecología general, unidos a los que determinan sus patrones de distribución y entorno biogeográfico, así como los llevados a cabo sobre sus respuestas a factores de estrés como la contaminación orgánica, eutrofización, etc, permiten asegurar que se dispone de una importante fuente de información, con valor científico, para acreditar el uso de estos organismos en los programas de biomonitorización y de control de calidad del agua (e.g. Bunn and Davies, 2000; Norris and Hawkins, 2000; Wright et al., 2000; Bailey, et al., 2004; Hering, et al., 2004).

Sin embargo, muchos aspectos de la vida acuática están mediados y condicionados por el marco geográfico donde se desarrolla, incidiendo sobre ella el clima, la geología y la historia de cada región biogeográfica. Esto tiene especial significado y relevancia en la región mediterránea, donde se dan las situaciones y gradientes ambientales más extremos y contrastados (Gasith and Resh, 1999) dentro del ámbito europeo, y donde se usan de forma más o menos generalizada, índices e indicadores que, aunque con vocación generalista, deben ser adaptados y ajustados.

En este sentido, se enmarcan los objetivos del presente trabajo, que inciden básicamente sobre el conocimiento de distintos aspectos de la ecología de los invertebrados acuáticos de la Cuenca del Río Segura. Aún cuando se lleva más de 20 años prospectando y analizado de forma general, o parcial, muchas poblaciones y comunidades acuáticas en la Cuenca del Segura, no se dispone en la actualidad de estudios generales que abarquen, bien la cuenca en su totalidad, bien sus comunidades de invertebrados acuáticos, en general. Como

antecedentes, se cuenta con varios estudios que, de forma más o menos intensiva, analizan aspectos de la biología y/o ecología de diferentes grupos taxonómicos, en el ámbito geográfico de la Cuenca del Segura (sobre moluscos acuáticos: Gómez, 1988; Vidal-Abarca et al., 1991a; coleópteros: Gil, 1985; Gil et al., 1990; Millán, 1991; Delgado, 1992; Delgado et al., 1992; Millán et al., 1992; 1993; 1996; Sánchez-Meca et al., 1992; Delgado y Soler, 1997; Abellán, 2003; Sánchez-Fernández, 2003; 2004a; 2004b; Abellán et al., 2004; 2005; Sánchez-Fernández et al., 2003 ; heterópteros: Millán, 1985; Millán et al., 1989; plecópteros y efemerópteros: Ubero-Pascal, 1996; Ubero-Pascal et al., 1998; odonatos: Suárez et al., 1986 y tricopteros: Bonada et al., 2004). Algunos trabajos estudian las comunidades de invertebrados acuáticos en ámbitos geográficos más pequeños como en ramblas (Ortega, 1988; Ortega et al., 1991; Moreno, 1994; 2003; Miñano, 1994; Moreno et al., 1997; 1999; Guerrero et al., 1998), en pequeños ríos o afluentes secundarios del Río Segura (Suárez et al., 1983; 1986; Vidal-Abarca et al., 1991b; Guerrero, 1996; 2002; Guerrero et al., 1996; Ubero-Pascal, 2000), e incluso en sistemas leníticos de pequeñas dimensiones (Suárez et al., 1991; Gómez et al., 2002).

El único trabajo referido a la totalidad de la Cuenca del Segura, que analiza a escala global las comunidades de invertebrados acuáticos, es el de Mellado et al. (2002).

Esta falta de estudios básicos e integrados, es lo que ha llevado a la elaboración de la presente memoria que, en cuatro capitulo pretende aportar información general sobre distintos aspectos de la ecología de los invertebrados acuáticos de la cuenca del Río Segura, necesaria para utilizarlos como indicadores de la calidad el agua. Además, el estudio se incluye dentro de los objetivos del proyecto GUADALMED, en el que participan 6 universidades españolas y el CEDEX, generado para estandarizar y probar una

metodología apropiada a los ríos mediterráneos, en concordancia con los principios expuestos en la Directiva Marco el Agua (DMA) (ver Limnética, 2002).

Así en el primer capitulo, se analiza las posibles fuentes de variación (estacionalidad en la toma de muestras, método de procesado de las muestras, nivel de resolución taxonómica y tipo de datos: presencia-ausencia o abundancia relativa) que pueden dificultar o cuestionar la validez de los sistemas rápidos de evaluación biológica ("Rapid Bioassessment Protocols") en el ámbito mediterráneo, ejemplarizado en la Cuenca del Río Segura. Estos sistemas, que utilizan a los invertebrados acuáticos como detectores de la calidad del agua, además de ser más rápidos que los tradicionales, tienen la ventaja de ser menos costosos y, en definitiva más apropiados para su uso por la administración pública en el control de la contaminación (y otros impactos) de los cauces, aunque están sujetos a numerosas críticas (e.g. Doberstein et al., 2000; Humphrey et al., 2000; Lenant and Resh, 2001; Reece et al., 2001).

El segundo capítulo pretende realizar una tipificación de los ríos de la Cuenca del Segura, en función de las comunidades de invertebrados acuáticos que los habitan e indagar en los parámetros ambientales (naturales y antrópicos) que explican, a gran escala, su distribución.

El tercer capítulo profundiza en los caracteres o rasgos diferenciales de las especies (*species traits*) que componen la comunidad de invertebrados acuáticos de la Cuenca del Río Segura, en un intento por definir las diferencias en la composición y estructura de las comunidades de invertebrados que se detectan en ríos de distinta topología. Además, y de forma innovadora se utiliza por primera vez en ríos, un análisis multivariante (RLQ análisis: Dolèdec et al., 1996) que soluciona el problema de relacionar dos conjunto de datos (en nuestro caso dos tablas, una construida con características ambientales, y otra con los

caracteres o "traits" de las especies) con un tercero que relaciona las anteriores (en nuestro caso una matriz de abundancia de especies).

Por último, en el cuarto capítulo, se explora el papel que puede tener la diversidad funcional, en el sentido de Champely and Chessel (2002), para su uso como indicador de la calidad ecológica de los ecosistemas acuáticos. En este sentido, el uso de los caracteres funcionales de las especies que constituyen la comunidad de macroinvertebrados acuáticos, en vez de su riqueza u otro índice tradicional de diversidad (como el de Shannon o el de Simpon, etc), parece una buena herramienta para detectar cambios en la calidad ecológica de los sistemas fluviales, habida cuenta de que los impactos humanos sobre los cauces, en primera instancia afectan a la biodiversidad.

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Chapter 1

Macroinvertebrate assessment in streams from the Segura River basin (SE Spain): Seasonal trends, processing method and taxonomic resolution effects on multivariate patterns and community metrics.

Chapter 1. Macroinvertebrate assessment in streams from the Segura River basin (SE Spain): Seasonal trends, processing method and taxonomic resolution effects on multivariate patterns and community metrics.

Abstract

Aquatic macroinvertebrate samples were taken seasonally from 11 streams from the Segura river basin (SE Spain) from 1999 to 2001 to detect temporal patterns in communities that could lead to differences in bioassessment results. Sites belonged to four contrasting stream types. Two sorting methods were used. Firstly, macroinvertebrate samples were live-sorted in the field. Then, a whole sample was collected from each site and subsampled in the laboratory with a fixed-count method (200 individuals). Multivariate analyses were applied to detect changes in community structure caused by seasonality, sorting method, taxonomic resolution and data type (binary versus relative abundance). We also used a multivariate analysis of variance to look for differences in community metrics between sorting methods, seasons and stream types. Multivariate analyses did not show seasonal discrimination of the samples and single-seasons models were fairly similar. Live-sorting resulted in better discriminations between stream types than laboratory subsampling. Family level identification provides comparable results as the genus level at a broad environmental scale, while genus identification performed better detecting more subtle differences. Relative abundance provided better results than binary data, although differences were almost negligible at the genus level. Analysis of variance did not detect differences in community metrics among seasons and differences among stream groups were all significant. Almost all metrics tested showed significant differences between sorting methods, with higher values obtained for live-sorting. Our study has important implications for stream bioassessment in our region.

1. Introduction

The evaluation of water quality by means of biological parameters has been widely used over the last century. The high cost of quantitative approaches has led to the development of semi-quantitative methods called Rapid Bioassessment Protocols (RBPs) (e.g. Plafkin et al., 1989). The original purpose of using RBPs was to identify water quality problems and to document long-term regional changes in water quality and their chief advantage is the reduction of the intensity of study required at individual sites which permits a greater number of sites to be examined (Resh and Jackson, 1993). These semi-quantitative approaches have statistical implications because the lack of replicates for one site (and one date) eliminates some classical parametric methods from being used. However, the "reference condition approach" (Reynoldson et al., 1995, 1997; Wright, 1995), which uses semi-quantitative sampling and multivariate statistics, circumvents many of the problems inherent in quantitative, inferential approaches (Reynoldson et al., 1997).

On the other hand, seasonal variations are well documented to occur in stream macroinvertebrate communities. Studies on headwater streams have shown a seasonal sequence of species replacement and quite characteristic seasonal cycles in community structure and function (Giller and Malmqvist, 1998). These changes can be relatively marked in some systems (Furse et al., 1984; Feminella, 1996) or can be weaker (Death, 1995; Zamora-Muñoz and Alba-Tercedor, 1996). Macroinvertebrate life cycles, seasonal changes in environmental variables (Hawkins and Sedell, 1981) and discrete disturbance events (Fisher et al., 1982; Boulton and Lake, 1992) that differentially affect taxa in a community are responsible for those changes. Seasonal variations can affect both biotic integrity metrics (Murphy, 1978) and the performance of multivariate predictive models

(Linke et al., 1999; Murphy and Giller, 2000; Reece et al., 2001), although other studies have shown relative stability of particular biotic indices or multivariate results through time (Zamora-Muñoz et al., 1995; Zamora-Muñoz and Alba-Tercedor, 1996).

On the other hand, a sampling methodology that markedly focuses on getting the maximum diversity (versus one that aims to estimate abundance patterns) may find less marked seasonal changes if shifts in abundance are more common than replacement of species. Similarly, the particular habitat sampled may affect the temporal patterns observed because of the appearance or exclusion of habitat-specific taxa or movements between habitats coupled with seasonal changes in resources.- e.g. the habitat availability in intermittent streams, where some rheophilic taxa can migrate from drying riffles to pools (Chessman, 1999) or simply disappear (Brunke et al., 2001). Recognizing the influence of sampling and/or sorting methodologies on the observed temporal variability of community structure would improve the quality of models, as has been addressed recently (Humphrey et al., 2000).

Another key element in the application and performance of RBPs is the sample processing. Most of the approaches involve a subsampling process with relatively constant effort (Resh et al., 1995) while subsampling strategies vary between protocols. United Kingdom authorities (Wright, 1995) sorted samples in the laboratory in a standardized manner for approximately 2 h, Parsons and Norris (1996) used laboratory subsampling to 200 individuals, while other protocols involve a subsampling procedure of picking live animals in the field for a set period or to a set number (e.g. Lenat, 1988; Chessman, 1995). In a broad between-agencies comparison in Australia, Humphrey and Thurtell (1997) found that live-sorting usually resulted in higher error rates than laboratory sorting, and Humphrey et al., (2000) concluded that this was due to an under-representation of taxa in

live-sorted samples derived from a) low sample sizes, b) operator inexperience and c) common taxa that were missed. They found that some small and cryptic taxa (along with some chironomid subfamilies) were usually missed from live-sorted samples, whereas large taxa were better represented.

There is also controversy about fixed-count subsampling procedures. While some authors defend the fixed count methods (Barbour and Gerritsen, 1996; Somers et al., 1998), others argued that such methodologies introduce bias that may compromise bioassessment results (Courtemanch 1996; Doberstein et al., 2000), particularly because of the sample size effects in taxa richness and related measures.

Taxonomic resolution is another source of variation in detecting community patterns. While some studies have shown little or no differences in multivariate bioassessment results (Bournaud et al., 1996; Bowman and Bailey, 1998; Bailey et al., 2001), other authors recommend the identification to species or genus (Guerold, 2000; Lenat and Resh, 2001), or combined genus and species level for certain groups as Chironomidae (King and Richardson, 2002). On the other hand, presence-absence data offer potential time-cost savings and has yielded multivariate results comparable to abundance data in several studies (Furse et al., 1984; Thorne et al., 1999).

The Water Framework Directive (European Commission, 2000) requires that the European countries need to assess the ecological status of their freshwater ecosystems using biological indicators, and to achieve the "good ecological status" by 2015. Therefore, there is an urgent need to establish standard methodologies to assess the biotic integrity of aquatic ecosystems as there are in other countries. In this context, we tried to establish a common protocol to measure the ecological status of Mediterranean basin streams (Prat,

2002). As part of this larger study (that also included water chemistry measures, in-stream habitat characterization or riparian forest assessment) we collected macroinvertebrate samples from 18 minimally-impacted sites in the Segura River basin (SE Spain) on seven occasions from 1999 to 2001 to account for natural seasonal variations in community structure and biotic integrity metrics. Eleven of these sites were sampled using two methods: In the first one, invertebrates were live-sorted in the field trying to collect the highest possible diversity by actively searching for rare taxa. In a second protocol, a multi-habitat composite sample was subsampled in the laboratory using a fixed-count (200 individuals) plus a subsequent search of large and rare taxa (LR search procedure, Courtemanch, 1996; Vinson and Hawkins, 1996). We present here the results from the application of both processing approaches to compare descriptions of communities. Furthermore, we sought possible seasonal changes in invertebrate communities that could lead to undesirable "noise" in biomonitoring results. Also, we focused on the effect of taxonomic resolution (genus *vs* family) and the nature of the data (presence-absence *vs* percentage abundance) on multivariate results. In the majority of multivariate approaches to bioassessment, classification and ordination techniques are used to classify and spatially plot reference (usually minimally-impacted) sites of known characteristics and then compare their position relative to unknown quality test sites. We included in our study four stream groups or types of contrasting macroinvertebrate communities to test how the different factors affect the multivariate ordination models and discuss the possible implications in bioassessment. Our specific questions were:

1. Is the live-sorting methodology useful in terms of providing more information about community structure than the laboratory sorting of organisms, thus increasing the discrimination among stream types and/or seasons?

2. Is it also more effective in recovering a higher number of taxa than the laboratory subsampling? And also, is our method biased towards large and against small-cryptic animals?

3. Does genus identification offer a better explanation of the variability in community patterns (spatial stream types and temporal seasonal differences) than the family level?

4. Do ordinations based on percentage abundance data better discern among stream types than presence-absence data?.

5. Do biotic integrity metrics vary among stream types and seasons? Do they vary with sample processing method? Is there more variation among methods than among sites?

2. Methods

2.1 Study area and sampling sites

The study sites are located in the Segura River catchment, SE Spain (Figure 1). General descriptions of the basin area (geology, climate, etc.) can be found elsewhere (Vidal-Abarca et al., 1992; Mellado et al., 2002). We conducted our study in 11 streams belonging to 4 different typologies: 5 streams in forested mountainous areas, 3 streams located at medium altitude semi-agricultural areas, 1 spring-fed stream at low altitude, and 2 semiarid naturally saline streams. These sites were known to harbour different communities from previous studies (Mellado et al., 2002, Millán et al., 1997; Moreno et al., 1998; Ubero-Pascal et al., 1998; Vidal-Abarca et al., 1990) and so, this stratified sampling design was supposed to give

a background discrimination to be tested by means of multivariate analysis and community metrics.

Sites 1, 2 and 3 were 3rd and 2nd order, high altitude reaches (950 to 1020 m.a.s.l.) of the Segura, Madera and Taibilla streams respectively, located in mountainous conifer-forested areas inside or very near a nature reserve, in the humid NW part of the basin. Sites 5 and 18, Mundo and Zumeta streams, are 4th order, medium altitude (650 and 720 m.a.s.l.) stream reaches and like sites 1, 2 and 3, are
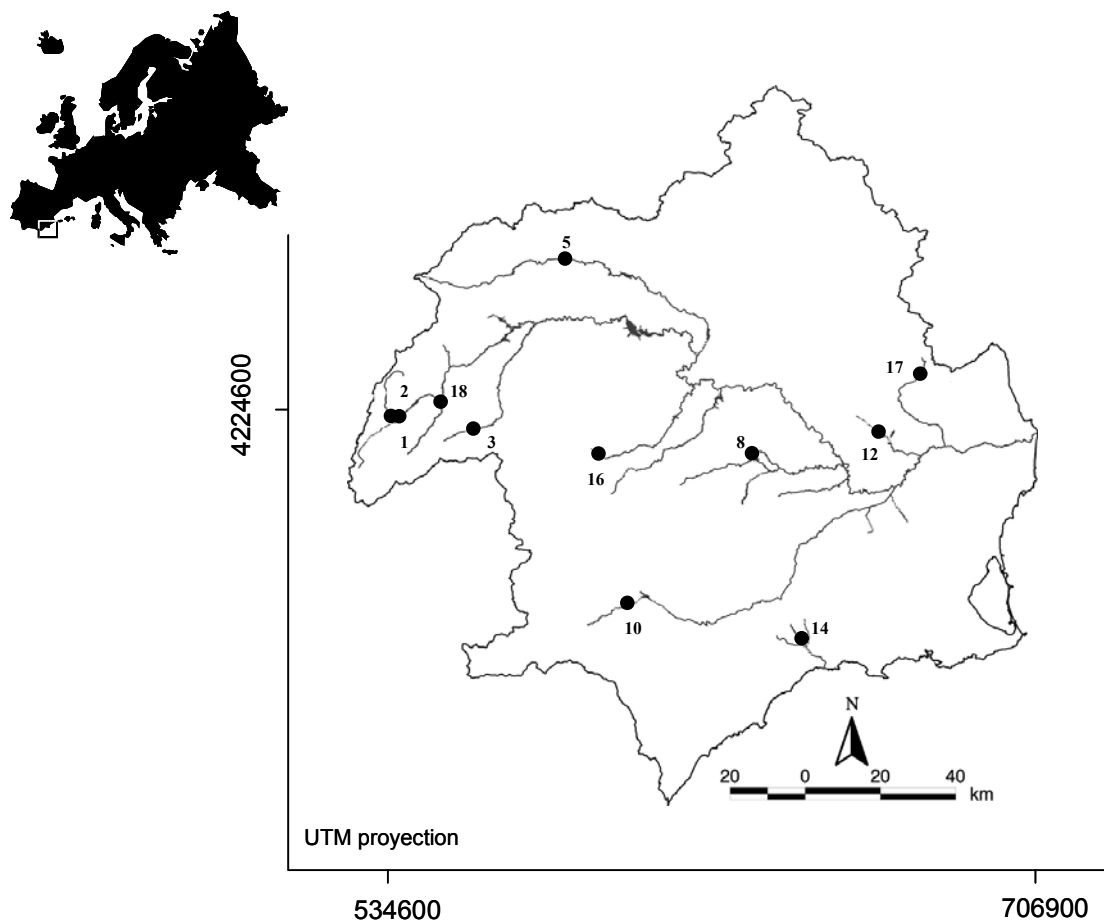
Figure 1. Study area and sampling locations.

located in forested areas in or near the nature reserve. We will call this group as "Mountain Streams" (MS).

Sites 8, 10 and 16, Fuente Caputa, Corneros and Argos streams, are also medium altitude (420, 650 and 780 m.a.s.l. and 1st , 3rd and 4th order) reaches located in extensive semi-agricultural catchments from the less humid middle of the Segura river basin, thus affected in some degree by agriculture runoffs, water diversions for irrigation, livestock grazing or recreational activities. We called this group "Semi-Agricultural Streams" (AS).

Site 17, "Chicamo stream", (ChS) is a 1st order spring-fed stream at low altitude (340 m.a.s.l.) with some signs of impairment due to agricultural, livestock grazing and recreational activities. It is also affected by water diversions for irrigation. Site 17 has a depauperate invertebrate community. We considered this site as a different stream type because of its poor invertebrate community and could be considered an impacted site.

Finally, Sites 12 and 14 (Rambla Salada and Rambla del Reventón) are low altitude (100 and 140 m.a.s.l.) saline streams (average salinities about 15 and 40 g/L respectively) located in semi-arid areas of the catchment. Some agricultural impacts may affect these reaches, although their naturally high salinity is responsible for their low taxa richness. We named this group "Saline Streams" (SS).

2.2 Macroinvertebrate sampling and processing

134 macroinvertebrate samples were taken from the 11 streams in 7 occasions from 1999 to 2001: April 1999, July 1999, November 1999, February 2000, April to May 2000, July 2000 and December 2000 to February 2001. Some sites were not sampled in all 7 dates.

One single multi-habitat semiquantitative kick-sample, as described by Zamora-Muñoz and Alba-Tercedor (1996) was taken in each sampling occasion. In our sampling method, macroinvertebrates are live-sorted in the field from white trays with the aid of a portable aspirator trying to collect a representation of the community and getting the maximum diversity as possible, actively searching for rare taxa. The sampling goes on until no new taxa (at family level) are found in the field with successive trays. We preserved this field live-sorted subsample in 70% ethanol.

Another multi-habitat kick-sample was preserved in 1 L plastic jars. This sample was processed in the laboratory using a fixed count subsampling procedure (approximately 200 individuals when achievable) under a 5X magnification lens. Invertebrates were identified in the laboratory with the aid of a 6.5-64X Olympus microscope to the lowest taxonomic level (usually genus) except for some dipterans that were identified to families, subfamilies or tribes, Hirudinea (identified to family), Hydracarina, Tricladida, Oligochaeta, Nematoda, Ostracoda, Copepoda and Cladocera. For convenience, we use the term "genus" when referring to the identification level described above.

2.3 Data análisis

2.3.1 Multivariate analysis

We constructed eight data sets combining the factors we wanted to compare: processing method (live-sorting –LivS- versus laboratory sorting –LabS-), taxonomic resolution (genus versus family) and data type (presence-absence versus percentage abundance data). Data were transformed to percentage abundance due to the semi-quantitative nature of the sampling method. Relative abundance data were 4th-root transformed as recommended by

horne et al. (1999) for an effective discrimination of sites over a wide range of water quality.

We carried out a series of multivariate analyses for each data set and compared their results to investigate the effects of seasonality, sample processing method, taxonomic resolution and data type on the performance of each multivariate model. Firstly, analysis of similarities (ANOSIM, Clarke, 1993) was performed on Bray–Curtis similarity distances to test for differences between stream types and seasons. We used a two-way crossed ANOSIM with stream type and season as four level factors. Each test in ANOSIM produces an R-statistic, which contrasts the similarities among samples (our replicates) within a group (stream types or seasons in our case) with the similarities among samples between groups. R will take values near 1 when the similarities between samples within groups are higher than those between samples from different groups, and values near -1 in the opposite case. Values close to 0 are indicative of no differences among groups. Monte Carlo permutations number was set at 999. Significant ANOSIM results should be cautiously interpreted as means can be minimally different with much overlap in values among sample groups yet still produce statistically significant differences. Nevertheless, we used comparisons of the R-statistic, which has an absolute interpretation of its value and is not unduly affected by the number of replicates in each group (Clarke and Gorley, 2001), to compare models' ability to differentiate groups. As a general guide, R values can be categorized into 3 broad groups (Clarke and Gorley, 2001): 1. R > 0.75: indicates that there are large differences and the treatments/groups are well separated; 2. R > 0.5: indicates clear differences, but the treatments/groups are 'overlapping'; 3. R < 0.25: indicates little/no difference and the treatments/groups are barely separable. When ANOSIM results were significant, we also calculated ANOSIM pair-wise comparisons among stream types and/or seasons to distinguish among possibly contrasting effects.

Secondly, we use non-metric multidimensional scaling (MDS, Kruskal and Wish, 1978) to spatially plot the samples. Non-metric multidimensional scaling maps the samples in ordination space such that the rank order of the distances among samples on the plot matches their Bray–Curtis similarities, and samples that share similar assemblage composition will group together. To measure the effectiveness of two-dimensional MDS ordination plots in preserving the sample relationships Bray-Curtis similarity ranks, the stress S value (running 100 iterations) was included in each plot (S<0.20 is considered acceptable, Clarke and Warwick, 1994). Then, we constructed a 95% Gaussian bivariate probability ellipse (Altman 1978) around the mountain streams (MS) samples in each of the MDS plots and calculated the percentage of samples belonging to other stream groups that fell outside of the MS ellipse as a measure of the discriminatory power of each ordination model. This procedure is based on the last step of the BEAST (Benthic Assessment of SedimenT) bioassessment method (Reynoldson et al., 1995, 1997, 2001; King and Richardson, 2002).

To visualize the congruity among results of the eight analyses, a "second stage" MDS procedure was performed based on the 8 previously obtained similarity matrices. In this analysis, Spearman rank correlations (ρ) are calculated between each pair of matrices, being the resulting correlations matrix the base for a new MDS with the original matrices as elements of the ordination and the ρ coefficient the new "similarity measure". The closer appear two analyses, the more similar their results are.

The *PRIMER* v5 package (Plymouth Routines in Multivariate Ecological Research, Clarke and Gorley, 2001) was used to perform all multivariate analyses, while probability ellipses were constructed using *STATISTICA* v5.0 software package (Stat Soft Inc, 1995).

Seasonal variation should be manifest primarily at the genus level because representatives of families are likely to be present throughout the year, so again, one-way ANOSIM tests between stream types were applied for each of three single-season models using genus identifications, presence absence and fourth-root transformed relative abundance data and the Bray-Curtis similarity index for these analyses. Comparison of the *R* value served again as a measure of the goodness of fit of the models.

2.3.2 Comparison of community metrics

We applied the Iberian Biomonitoring Working Party (IBMWP, formerly BMWP') (Alba-Tercedor and Sánchez-Ortega, 1988; Alba-Tercedor and Pujante, 2000; Alba-Tercedor et al., 2004) biotic index and its relative IASPT (Iberian Average Score per Taxon) as biotic integrity indices. The IBMWP index is based on the British BMWP (Armitage et al., 1983) and it was adapted to the Iberian macroinvertebrate fauna by adding some families and modifying some scores. The IASPT index is calculated by dividing the IBMWP from a sample by the number of IBMWP families (only those considered in the index) in this sample.

As diversity measures and particularly richness metrics are known to have high sensitivity to sample size (Magurran, 1988; Gotelli and Colwell, 2001; Metzeling and Miller, 2001), we calculated the expected richness (for a simulated 100 individual sample, ES(100)) for each sample using rarefaction (Simberloff, 1978). In addition we constructed individual-based rarefaction curves using Ecosim software (Gotelli and Entsminger, 2001) and calculated the Abundance-based Coverage Estimate of species richness (ACE, Chao et al., 1993) using EstimateS software (Colwell, 2000) for both live-sorting (LivS) and laboratory subsampling (LabS) data sets. Differences in the IBMWP and IASPT biotic indices, family richness (number of families), genus richness (number of genera), rarefied genus richness,

EPT richness (number of taxa belonging to Ephemeroptera, Plecoptera and Trichoptera) and rare taxa richness (number of singleton taxa per sample) between different processing methods, seasons and stream types were assessed by a three-way multivariate analysis of variance (MANOVA). To test for the influence of macroinvertebrate size on the performance of the processing methods, we calculated two new richness variables: the number of taxa with a maximal size of more than ca.10 mm. and of less than ca. 2 mm. We compared these two variables plus the number of chironomid subfamilies between the two processing methods by ANOVA.

We inspected metrics for normality using normal-probability residual plots and tested variance homogeneity using Bartlett's test (p <0.05). All data met the assumptions of normal residuals, and all but EPT richness, met the assumptions of homogeneity of variance (Bartlett's test, $0.088 < p < 0.95$). EPT richness was log(x+5)-transformed and tested again (Bartlett's test, p=0.71). When MANOVA showed significant differences, Scheffe *a posteriori* tests were used to determine which stream types were significantly different at the 0.05 probability level. The ANOVA-MANOVA subroutine of the *STATISTICA* v5.0 software package (Stat Soft Inc, 1995) was used to run these analyses. We calculated the expected rarefied richness, ES(100), using the DIVERSE subroutine on PRIMER v5 (Clarke and Gorley, 2001).

3. Results

A total of 38638 organisms was processed, belonging to 23 orders, more than 100 families and around 275 taxa. Insects comprised 66.6 % of total abundance and 87.6 % of total number of taxa. Within the insects, the most abundant orders were dipterans (38.4 % of insect abundance), Ephemeroptera (19.5 %) and Trichoptera (9.7 %). Diptera was the most

diverse order with 60 taxa (22 % of total taxa richness), followed by Coleoptera with 55 taxa (20 %), and Trichoptera with 41 taxa (15 %). Overall, the most abundant taxa were the amphipod *Echinogammarus* (with 5875 individuals) followed by the midge subfamily Orthocladiinae (2836), ostracods (2170), and the mayflies *Caenis* (1664) and *Baetis* (1526). Mean live-sorted sample abundance was 351.2 (Range= 27-1133; SD= 204.7;N=67) while mean laboratory abundance was 222.3 (Range=159-450; SD=54.89; N=67).

3.1 Multivariate análisis

The 2-way ANOSIM results for the season factor were always not significant, with Global *R* values near to zero in all analyses, indicating no differences in community structure between seasons (Table 1). As predicted, there were significant global differences among stream types in all eight combinations of processing methods, taxonomic levels and data types considered (Table 1). Pair-wise comparisons among the 4 stream types were always significant too with lesser values of Global *R* between the mountain and the semi-agricultural streams groups (Table 1). Focusing on the effects of the sampling-processing on the multivariate patterns, we obtained higher values of Global *R* for the live sorted (LivS) samples, whatever taxonomic level or data type was considered. Taxonomic resolution influenced ANOSIM results in both processing methods, increasing the value of Global *R* mostly when using presence-absence data. Using the family resolution, we observed marked increases in Global *R* when switching from presence-absence data to relative abundance data, despite this increase being bigger for the laboratory processing (LabS) data set. However, when considering the genus level, increases in Global *R* using relative abundance instead of binary data were almost negligible in both cases. As pointed out above, pair-wise comparisons were significant in all cases. R values ranged from 0.5 to 0.7 for MS-AS comparisons and were close to 1 in the other cases (Table 1).

Table 1. ANOSIM *R* and associated probability values p obtained for the different analyses

2-way ANOSIM between Stream Types and Seasons and pair-wise comparisons between stream types																	
			Global ANOSIM				**ANOSIM Pair-wise comparisons between stream types**										
Sources of variation			**Stream Types**		**Seasons**		**MS, AS**		**MS, SS**		**MS, ChS**		**AS, SS**		**AS, ChS**		**SS, ChS**	
Method	Taxonomic level	Data type	Global R	p (%)	Global R	p (%)	R	p (%)	R	p (%)	R	p (%)	R	p (%)	R	p (%)	R	p (%)
LabS	Family	**Pres/Abs**	0.719	0.1	0.025	28.1	0.481	0.1	0.917	0.1	0.945	0.1	0.705	0.1	0.723	0.1	1,000	0.1
		4th-root	0.764	0.1	0.027	26.0	0.512	0.1	0.954	0.1	0.952	0.1	0.862	0.1	0.743	0.1	1,000	0.1
	Genus	**Pres/Abs**	0.789	0.1	-0.008	53.0	0.554	0.1	0.975	0.1	0.953	0.1	0.901	0.1	0.846	0.1	0.984	0.1
		4th-root	0.792	0.1	-0.002	49.9	0.545	0.1	0.981	0.1	0.928	0.1	0.963	0.1	0.776	0.1	1,000	0.1
LivS	Family	**Pres/Abs**	0.796	0.1	0.061	0.95	0.560	0.1	0.984	0.1	0.992	0.2	0.905	0.1	0.858	0.1	0.981	0.1
		4th-root	0.824	0.1	0.057	1.08	0.607	0.1	0.991	0.1	0.986	0.1	0.940	0.1	0.872	0.1	0.984	0.1
	Genus	**Pres/Abs**	0.853	0.1	0.037	2.02	0.660	0.1	0.994	0.1	0.984	0.1	0.936	0.1	0.928	0.1	0.984	0.1
		4th-root	0.856	0.1	0.045	1.93	0.657	0.1	0.996	0.1	0.978	0.1	0.963	0.1	0.934	0.1	0.984	0.1

Notes:
LivS=Live-sorting; LabS= Laboratory subsampling;
Pres/Abs, presence-absence data;
MS, mountain streams; AS, semi-agricultural streams; SS, saline streams; ChS, Chicamo spring.

When testing for the seasonal effect on the ability to discriminate among our stream types, we obtained comparable results for the three single-season models constructed with the LivS data, with similar ANOSIM R values than that obtained for the combined models (Table 2). However, the LabS spring model showed a lower discrimination power than the LabS summer and winter ones.

MDS plots and the BEAST approach to measure the discriminatory power of the ordinations to discern among stream types reflected similar trends as ANOSIM tests. Saline streams (SS) and Chicamo spring (ChS) were the best differentiated clusters, whereas Mountain Streams (MS) and semi-Agricultural Streams (AS) showed slight overlap, with some AS samples the only ones that fell into the MS 95% probability ellipses in all cases.

Stress values ranged from 0.14 to 0.16 (Figure 2). The models' discriminatory powers ranged from 71.8% to 84.6% for the LabS data and from 76.9 to 92.3% for the LivS data (Figure 2).

Globally, LivS models showed higher discriminatory power values than LabS models (average increase 5.1%). Considering the same taxonomic level and data nature, increases in discriminatory power were: 5.1 % for family-binary data, 2.6% for family-relative abundance data, 5.1% for genus-binary and 7.7% for genus-relative abundance data. Also, the effects of the taxonomic resolution (overall effect 11.5% increase in discriminatory power) were similar for both processing methods when considering presence-absence data (a 12.8% increase in discriminatory power by using genus instead of family level) but different when relative-abundance transformed data were used. For the LabS data, the increase in discriminatory power was 7.7% while for the LivS data it was 12.8%. The nature of the data (binary versus transformed relative abundance) had less influence on the discriminatory power of the ordinations than taxonomic resolution

(average increase 2.6%). A 5% increase in discriminatory power was achieved for the LabS data at the family level, while no effect was detected at the genus level. For the field-processed data, a 2.6% increase was detected at both taxonomic levels. To sketch out the influence of the data transformation, we performed the same 2-way ANOSIM tests for untransformed relative abundance data, ranging the R values from 0.488 to 0.593, around 25% lower values than R for 4th-root transformed or binary data.

The 2nd-stage MDS procedure showed two clear patterns (Figure 3): In a first axis (horizontal), the analyses were clearly separated by the processing method, being the LivS analyses in the left side of the MDS plot and the LabS ones in the right side. The second axis (vertical) separated the analyses mainly by the taxonomic resolution used, with the genus level analyses arranged in the upper part of the plot. Secondarily, the nature of the data was also discriminated in this vertical axis, with transformed relative abundance data approaches located above presence-absence ones, a trend much more marked for family than for genus taxonomic level, for which the nature of the data had very little influence.

Table 2. Between stream-types ANOSIM *R* and associated probability values p obtained for the three single-season and the combined models.

Comparison of three single-season models for ANOSIM "between stream types"

Seasonal ANOSIM:		Spring		Summer		Winter		All Seasons	
Method	Data type	Global R	p (%)	Global R	p (%)	Global R	p (%)	Global R	p (%)
LabS	**Pres/Abs**	0.713	0.1	0.845	0.1	0.807	0.1	0.789	0.1
	4th-root	0.724	0.1	0.836	0.1	0.801	0.1	0.792	0.1
LivS	**Pres/Abs**	0.833	0.1	0.865	0.1	0.867	0.1	0.853	0.1
	4th-root	0.863	0.1	0.879	0.1	0.846	0.1	0.856	0.1

Notes: LivS=Live-sorting; LabS= Laboratory subsampling.
Pres/Abs, presence-absence data. (Genus level data were used)
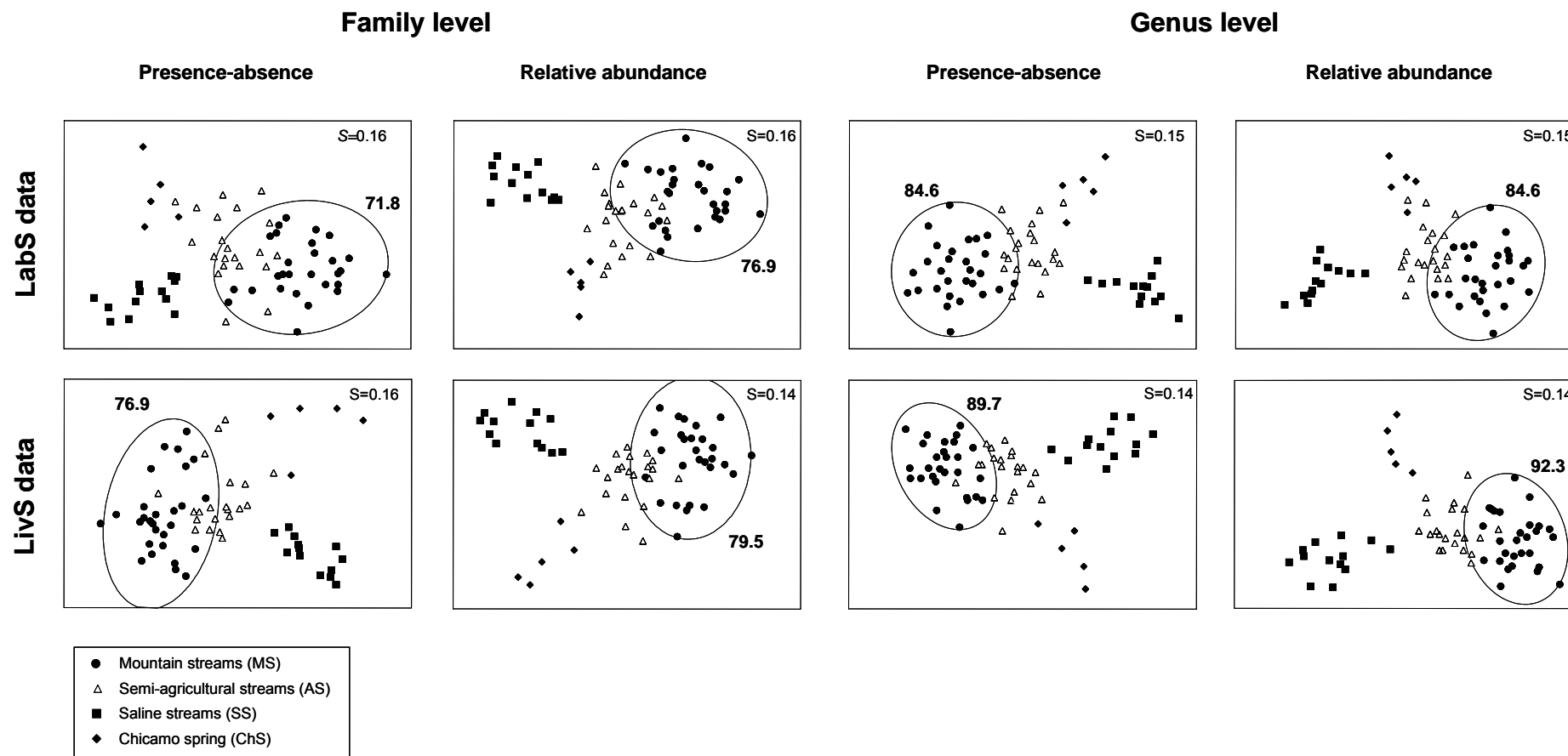
Figure 2. Non-metric multiple dimensional scaling (MDS) plots for each of the eight factors combinations. 95% probability ellipses are plotted around the mountain streams (MS) group. Stress value (S) is included in each ordination and the percentage accuracy or discriminatory power between MS and the other stream groups is written near the ellipses.
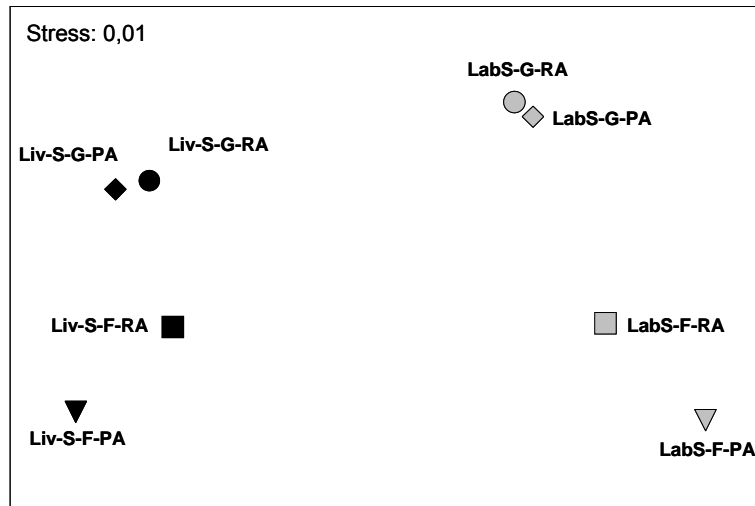
Figure 3. 2nd-Stage non-metric Multiple Dimensional Scaling (MDS) plot showing the relative positions of the different analyses evaluated. (LivS=live-sorting; LabS= laboratory subsampling; G=Genus level; F=Family level; PA=Presence-absence data; RA= 4th root-transformed Relative Abundance data)

3.2 Comparison of community metrics

MANOVA did not detect significant differences in any biological variable among seasons (Table 3). In contrast and as we expected, biotic variables were significantly different among stream groups (Tables 3 and 4). All metrics with the exception of the IASPT index showed significant differences between processing methods, with higher values obtained for the LivS method (Figure 4). We found also a significant interaction between *stream type* and *processing method* in family richness and genus richness, due to higher increases in richness from LabS to LivS in the more diverse communities (MS and AS streams) than in poorer ones (SS and ChS), where the LabS effort was enough to reach similar taxa numbers with LivS (Figure 5).

Rarefaction curves were different between methods, with LivS expected richness values always higher than LabS ones. The ACE estimate of asymptotic genus richness was also higher for the LivS data set (267 versus 227, Figure 6).We also found significantly higher number of taxa of both large (F=10.09; p=0.00197) and small (F=26.41; p=0.000001) organisms in LivS samples (Figure 4). The number of Chironomidae subfamilies was not different among methods (F=0.06; p=0.80).

Table 3. Multivariate analysis of variance (MANOVA) on community metrics. Factors and variables to which significant effects were found (p<0.05) are in bold.

MANOVA summary of all effects

Factors	Wilks' Lambda	Rao's R	df 1	df 2	p
Stream type (ST)	0.05125	23.86	21	276	0.0000
Season (SE)	0.74937	1.39	21	276	0.1216
Processing method (PM)	0.81328	3.15	7	96	0.0049
ST x SE	0.63989	0.72	63	546	0.9502
ST x PM	0.47622	3.88	21	276	0.0000
SE x PM	0.79877	1.07	21	276	0.3798
ST x SE x PM	0.63066	0.74	63	546	0.9311

Main effect: stream type					Main effect: season				
	MS Effect	MS Error	F (df 3,102)	p		MS Effect	MS Error	F(df 3,102)	p
Family richness	2484.546	35.429	70.13	0.0000	Family richness	24.556	35.429	0.69	0.5583
IBMWP index	98271.62	1277.962	76.90	0.0000	IBMWP index	651.696	1277.962	0.51	0.6763
IASPT index	20.88038	0.251	83.10	0.0000	IASPT index	0.342	0.251	1.36	0.2586
Genus richness	4686.726	81.148	57.76	0.0000	Genus richness	69.339	81.148	0.85	0.4674
EPT richness	5.194952	0.052	99.44	0.0000	EPT richness	0.056	0.052	1.06	0.3682
Rare richness	828.7542	15.015	55.20	0.0000	Rare richness	5.333	15.015	0.36	0.7855
Estimated richness ES(100)	2085.458	34.816	59.90	0.0000	Estimated richness ES(100)	91.103	34.816	2.62	0.0551

Main effect: processing method					Main effect: Interaction ST x PM				
	MS Effect	MS Error	F (df 1,102)	p		MS Effect	MS Error	F(df 3,102)	p
Family richness	624.594	35.429	17.63	0.0001	**Family richness**	107.582	35.429	3.04	0.0325
IBMWP index	10346.185	1277.962	8.10	0.0054	IBMWP index	2652.325	1277.962	2.08	0.1081
IASPT index	2.0262E-05	0.251	0.00	0.9929	IASPT index	0.155	0.251	0.61	0.6068
Genus richness	1305.895	81.148	16.10	0.0001	**Genus richness**	279.160	81.148	3.44	0.0196
EPT richness	0.329	0.052	6.29	0.0137	EPT richness	0.133	0.052	2.54	0.0605
Rare richness	79.115	15.015	5.27	0.0238	Rare richness	16.519	15.015	1.10	0.3527
Estimated richness ES(100)	289.054	34.816	8.30	0.0048	Estimated richness ES(100)	72.236	34.816	2.07	0.1082

Table 4. Means and standard deviations (SD) of community metrics within stream types. Means with different superscripts are significantly different at p<0.05 (Scheffe tests).

Variable:	FAM_R		GEN_R		ES(100)		EPT_R	
TYPE	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
MS	32.20[a]	7.78	42.75[a]	11.88	30.23[a]	7.40	11.89[a]	4.25
AS	29.11[b]	7.62	38.31[b]	11.71	26.23[b]	7.32	8.17[b]	3.15
SS	12.92[c]	4.25	17.12[c]	6.65	13.31[c]	5.20	1.54[c]	1.17
ChS	14.82[c]	4.19	16.88[c]	5.28	13.76[c]	4.49	3.29[d]	1.31

Variable:	RARE_R		IBMWP		IASPT	
TYPE	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
MS	15.09[a]	4.53	171.39[a]	47.07	5.53[a]	0.59
AS	12.51[b]	4.43	121.94[b]	35.08	4.43[b]	0.35
SS	4.00[c]	1.85	47.00[c]	18.96	3.66[c]	0.40
ChS	5.24[c]	2.05	60.53[c]	16.90	4.51[b]	0.46

Note: MS, mountain streams; AS, semi-agricultural streams; SS, saline streams; ChS, Chicamo spring.

FAM_R, number of families; GEN_R, number of gena; EPT_R, number of Ephemeroptera, Plecoptera and Trichoptera taxa;

RARE_R, number of singleton taxa.

4. Discussion

Among the most important technical issues for RBPs using macroinvertebrates are: seasonality, sampling methodology, subsampling and sorting and taxonomic identification level (Barbour et al., 1999). If we achieve a time-cost effectiveness by reducing effort in each one of these issues, we are then improving the RBPs applicability, which is the ultimate sense of such rapid approaches.

4.1 Seasonality

We did not find any pattern of seasonal trends that could have obscured the grouping of samples from the same stream typology. Seasonal samples from each of the stream types tended to be more similar to themselves than to any of the other stream types. Although
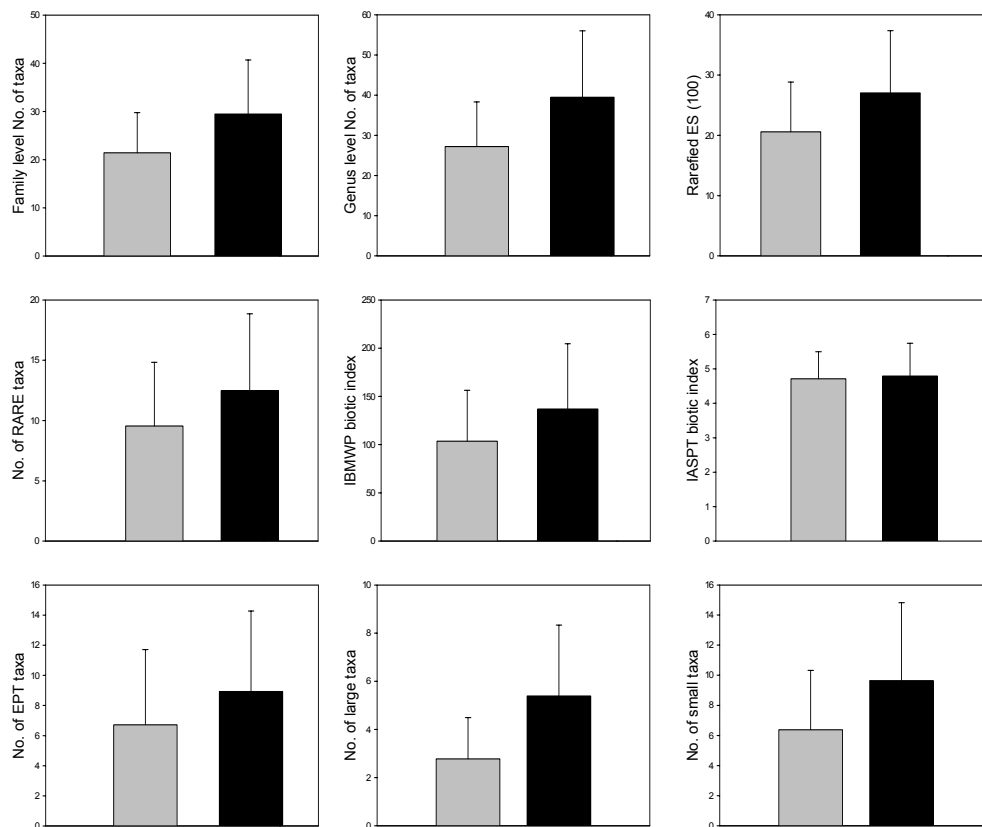
Figure 4. Comparison of community variables between laboratory subsamples (LabS, white bars) and live-sorted samples (LivS, black bars). All variables but IASPT showed significant differences at p<0.05 (MANOVA).
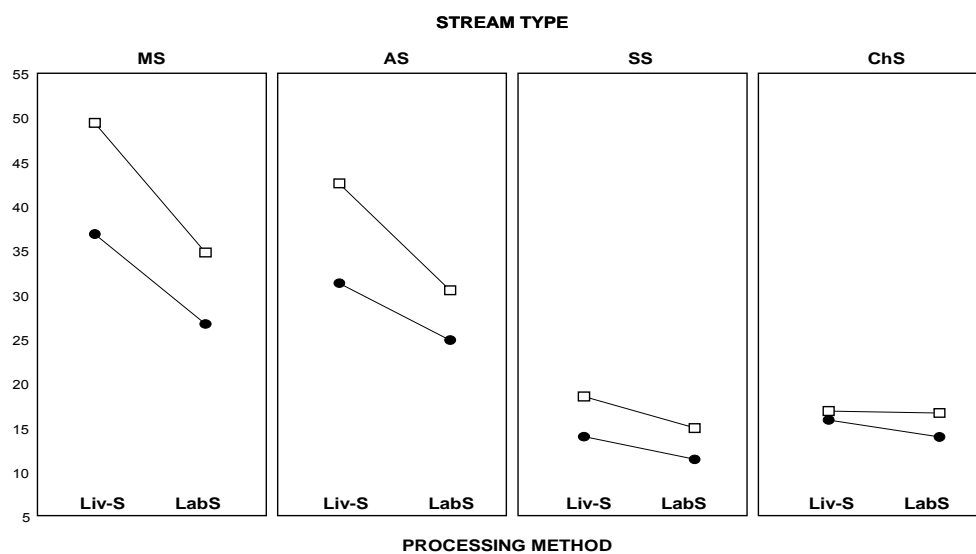
Figure 5.Mean family richness (black circles) and genus-richness (open squares) variations with processing method in each stream type (MS= mountain streams; AS= semi-agricultural streams; SS= saline streams and ChS= Chicamo spring). ANOVA *stream type* x *processing method* interaction was significant for family richness (F=3.04, p<0.05) and genus richness (F=3.44, p<0.05).
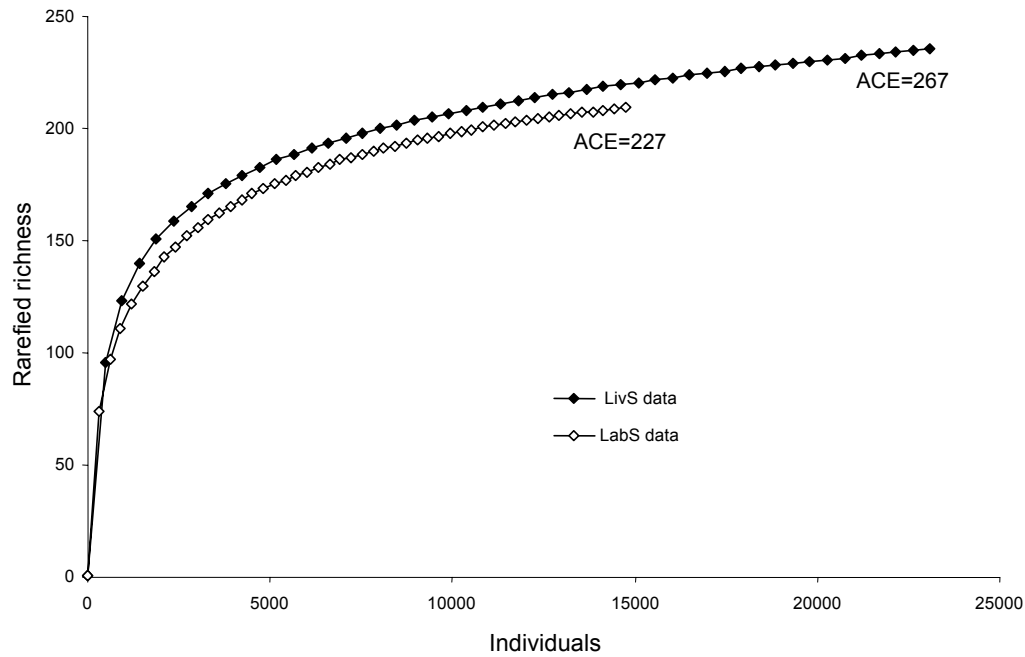
Figure 6. Individuals based rarefaction curves for laboratory subsampling (LabS) and live-sorting (LivS) data sets. Abundance based Coverage Estimate of richness (ACE) for each data set is included.

we couldn't test for seasonal differences within sites due to the lack of replication, additional ANOSIM tests within stream types (MS and AS, the most represented groups) did not reflect any seasonal pattern (these results have been omitted for brevity). In addition, when we compared 3 single-season models (spring, summer and winter) in differentiating stream groups, we did not observe strong differences among them when considering the field processed data. However, when looking at fixed-count laboratory sorted samples, some differences between single-season multivariate models appeared, with the spring model performing worst relative to summer and winter ones in detecting between-group differences.

MANOVA did not show seasonal differences for any of the variables considered. These results were consistent among processing methods and stream types. None of the interactions with the season factor were significant, supporting the results obtained in multivariate analyses and indicating the seasonal stability of the benthic macroinvertebrate

communities studied**.** These results are indicative of a temporal persistence in community patterns (at least at a reach spatial scale and a 2 years temporal scale) that would be required for bioassessment programs based on pattern detection (Bunn and Davies, 2000). Moreover, our results suggest the suitability of a single season approach for biomonitoring purposes in the study area, circumventing the need for seasonal corrections of biotic integrity metrics (Lenat, 1993; Whiles et al., 2000) or the use of single-season or combined seasons models (Reece et al., 2001) needed in other regions.

4.2 Processing method

Some RBPs involve a live-sorting methodology in which live organisms are picked from a tray just after sampling (Lenat, 1988; Chessman, 1995; Tiller and Metzeling, 1998; Metzeling and Miller, 2001). Some 23% of the monitoring programs in US used a live-sorting methodology, 75% of which focused on maximizing faunal richness (Carter and Resh, 2001). Although it had been argued that laboratory processing is desirable to standardize conditions and ensure randomness, the live-sorting is more time-cost efficient because picking up live animals is easier because movements help to find them. It is also more effective in terms of getting higher diversities because small and cryptic creatures can be seen better while moving. Moreover, the active searching for rare species increasing richness values which is critical for bioassessment purposes (Cao et al., 1998; 2001).

On the other hand, collecting animals separately while alive avoids the undesirable high amounts of organic detritus and sediment that occur when whole samples are taken, which means a better efficiency in terms of time in subsequent laboratory sorting. Moreover, the sampled area can be much wider and again the number of species collected. Additionally, using a portable aspirator helps to capture organisms too small and/or fragile to be picked

up with forceps, as ostracods, small snails or early instars insects, as well as to capture highly mobile animals as baetid mayflies or dytiscid beetles, circumventing the bias argued by Carter and Resh (2001) on rapidly moving taxa when sorting live material. The field live-sorting substantially minimizes the amount of preservative used, avoids the unintentional capture of organisms belonging to threatened species, and minimizes the possible sampling disturbance on communities, especially in small habitats. Finally, it offers the possibility of obtaining immediate field data sets (see *BioRecon* protocols in Barbour et al., 1999; Chessman, 1995) with moderately trained technicians (family level identification) that can be crucial for rapid bioassessment application.

Humphrey and Thurtell (1997) and Metzeling (2001) recommend a field sorting of at least 200 individuals for up to 60 minutes under the Monitoring River Health Initiative in Australia. Canada's EMAN protocols for measuring biodiversity suggest a 15-min sorting period or a target of 300 individuals (Rosenberg et al., 1997). We collected a mean number of organisms of approximately 350 organisms, and the time spent sorting a sample was around 40 minutes. We have tested the applicability and performance of a live-sorting field methodology and the results obtained are better than those achieved with a fixed count subsample of ca. 200 in the laboratory. ANOSIM significantly differentiated the stream types for both processing methods, however, values of the R statistic were always higher for LivS than for LabS analyses, and discriminatory power, as estimated by the BEAST ellipses method, was also greater using LivS data, with an average increase of 5.1%. Although it is not a marked improvement, it is an additional advantage of the use of the live-sorting methodology.

Differences in community metrics also illustrated the better performance of the field processing methodology. All metrics but the IASPT index showed significant increases

when applying the live-sorting methodology (Figure 4). The IASPT index is less affected by taxonomic richness than IBMWP, and thus it was not influenced by the processing method. The fact that not only the observed richness but also the rarefied expected richness showed a significant increase (Figure 4), demonstrate that the higher effectiveness of the live-sorting is affecting richness values, instead of being simply a sample size (number of organisms collected per sample) effect. This is illustrated by the rarefaction curves for both processing methods and the ACE expected richness values (Figure 6). Furthermore, the higher values found for the number of rare species (Figure 4) also suggested a better performance of the live-sorting procedure in maximizing biodiversity. The significant interaction found between stream type and processing method is interpreted as a higher reliance on the methodology in taxonomic richer environments. The IBMWP index is interpreted categorically in the following manner: scores of >100 (class I) are indicative of clean water, 61–100 (class II) of mild pollution, 36-60 (class III) polluted waters, 16-36 (class IV) very polluted waters and <16 (class V) strongly polluted waters. Considering IBMWP quality classes, 36% of the sites experienced a decrease in quality from LivS to LabS data showing that not only absolute values are affected by the sampling-processing methodology, but also the quality classes. This reinforces the idea that the correct application of this index must incorporate its original protocol.

Humphrey et al., (2000) found that live-sorting methods used by some water agencies of Australia were less successful in terms of number of taxa recovered than laboratory sorting ones. We have found an opposite trend, probably due to the nature of our sampling procedure: as we argued above, the sampled area for live-sorting, although not estimated, was probably wider than the area sampled to take the preserved sample. Another likely reason explaining the higher taxa richness in live-sorted samples is the use of the aspirator, much more efficient than forceps or wide mouthed pipettes in our opinion. Moreover, we

did not find any bias concerning the size of the organisms, having captured significantly more both larger (>10 mm) and smaller (<2 mm) organisms in live-sorted samples. The number of Chironomidae subfamilies neither was different among methods.

4.3 Taxonomic resolution and data type

Comparative studies of assemblages require decisions about the level of taxonomic resolution to which organisms are identified, and the biological attributes to be measured – presence-absence or abundance (Lasiak, 2003). If a comparative pattern persists at a lower taxonomic resolution (i.e. family level), then it is an advantage to use that lower level in terms of time and cost required to obtain meaningful information from a sampling program (Metzeling and Miller, 2001). Several analyses of benthic macroinvertebrate communities have shown little change in the multivariate description of community changes at different taxonomic levels (e.g. Furse et al., 1984, Bowman and Bailey, 1998). In our case, while the use of less precise taxonomic levels did not affect the significance of ANOSIM tests, the use of genus identifications was the main factor increasing ANOSIM R values and also the discriminatory power of the MDS-*BEAST* models. As we pointed out before, discriminatory power between MS and SS or ChS samples was in all cases 100%, which we can interpret as a separation of communities due to degradation or to extreme differential environmental features (ej. salinity). However, differences in model performance arose when comparing the ability to discriminate between moderately impacted semi-agricultural streams and mountain streams, both representing taxonomically richer ecosystems. In this case, the use of genus level resolution increased the accuracy by 11.5%, more than double that of processing method (5.1%) and four times more than data type (2.6%) on average. Bowman and Bailey (1998) also found that community descriptions based on genus-level identifications were less similar than descriptions based on family-

level identifications when the overall fauna was more diverse. Bailey et al., (2001) suggested a family-level broad bioassessment followed by a genus level identification if more precise information is needed. Our results would support this approach. On the other hand, the extent to which taxonomic aggregation affects multivariate analyses will also depend on the species distribution amongst higher taxa (Somerfield and Clarke, 1995), so generalizations should not be done about the correct resolution level to use before testing its effect on target communities.

Some studies have shown that spatial patterns of macroinvertebrate communities evident in quantitative data are generally preserved in binary data (Furse et al.,1984; Wright et al.,1995; Thorne et al., 1999). Our study supports these results, with little increases in ordination accuracy at the family level when transformed relative abundance was used and no influence at the genus level. A possible explanation is that when genus level is used, abundances are more equally distributed among taxa, with more low abundance or rare taxa. Thus, a relative abundance matrix is more similar to a binary one. In contrast, when aggregating genus into families, percentages of abundance will change because of the differential distribution of genera within families so differences with the presence-absence data matrix will increase too. Thorne et al. (1999) recommended a severe down-weighting of abundant taxa when comparisons are being made between relatively unpolluted sites with high richness. Binary data and fourth root-transformation represent such severe down-weighting of abundant taxa. 2-way ANOSIM tests for untransformed data resulted in R values ranging from 0.488 to 0.593, around 25% lower values than R for 4th-root transformed or binary data, so our results strongly support adopting this data transformation. Nevertheless, the small increase in performance associated with the high effort of counting individuals lead us recommend the use of binary data for bioassessment purposes. On the other hand, family and genus numbers of taxa showed similar trends

between stream types, seasons and processing methods, probably due to the great number of families containing low numbers of genus in our data set (86 families with 4 or less genus versus only 16 with 5 or more).

Our study has four important implications: 1) A single season sample provides enough information to detect patterns of change in macroinvertebrate communities in our region, 2) The live-sorting procedure performed better than the laboratory fixed-count subsampling and did not show size-dependent bias, 3) Family level identification provides comparable results to the genus level at a broader environmental scale, while genus identification performed better at detecting subtle differences within communities, and 4) Presence-absence data can explain the majority of variation in community structure.

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Chapter 2

Macroinvertebrate communities from the Segura River basin (SE Spain): stream types, indicator taxa and environmental factors explaining spatial patterns.

Chapter 2. Macroinvertebrate communities from the Segura River basin (SE Spain): stream types, indicator taxa and environmental factors explaining spatial patterns.

Abstract

Macroinvertebrate assemblages are largely determined by environmental factors, the assemblage structure representing a combination of environmental measures. In this work, we tried to reveal the main environmental trends affecting the broad-scale macroinvertebrate distribution in streams from the Segura river basin by means of multivariate analyses. We first classified streams according to their macroinvertebrate assemblages, describing groups in terms of environmental features. Then, we tried to find the main indicator taxa for each one of the identified stream types. Finally we investigate the links between the environmental factors and the biotic component. Our site classification (and a posteriori ordination) resulted in four main distinguishable classes, clearly positioned in a distinct portion of the longitudinal gradient: headwaters, middle courses and lower courses. Another group was composed of tributaries flowing into middle courses of the main rivers. Faunal composition and community structure was markedly distinct among the classified groups. We identified indicator taxa for each of the stream groups, predominantly aquatic Coleoptera. Salinity and pollution appeared as the keystone factors governing broad-scale macroinvertebrate distribution patterns in the Segura River basin. This main gradient was inversely correlated with the catchment longitudinal gradient, from freshwater, pristine, high altitude, humid headwaters in the NW to polluted lower reaches towards the more arid SE. This inter-correlation between natural variations and human-caused stress variables makes difficult to disentangle the array of confounding factors that influence community patterns in the Segura river basin.

1. Introduction

Stream macroinvertebrate communities vary spatially and temporally (Minshall and Petersen, 1985; Boulton and Lake, 1992; Townsend and Hildrew, 1994), and largely in relation to environmental factors (e.g. Brewin et al., 1995; Leland and Fend, 1998). Abiotic factors that influence community structure differ from system to system and with the spatial scale (Tate and Heiny, 1995; Vinson and Hawkins, 1998; Boyero, 2003). Environmental conditions that have been associated with community structure include water chemistry, altitude and temperature, discharge, substrate, riparian vegetation, land use, etc. Current velocity and depth are also major determinants of invertebrate communities in streams (Hynes, 1970; Allan, 1995; Degani et al., 1993), and stream width have also been reported as a key factor structuring invertebrate communities (Heino et al., 2003; Paavola et al., 2003). So, macroinvertebrate assemblages are largely determined by environmental factors, the assemblage structure representing a combination of environmental measures. This simplifies the identification of stream types and detection of changes in environmental condition by monitoring macroinvertebrates communities and thus providing a way to better manage water resources. Macroinvertebrate assemblages have been used to classify streams in groups of contrasting environmental conditions. The term "classification" implies that sets of observations or characteristics can be organized into meaningful groups based on measures of similarity or difference (Gauch, 1982). Stream classification is essential for understanding the distribution of ecological patterns within drainage networks and for developing management strategies that are responsive to the ecological patterns (Naiman 1998). Classification of stream ecosystems using macroinvertebrate communities and multivariate techniques has been widely investigated (e.g. Hawkes, 1975; Ormerod and Edwards, 1987; Naiman 1998; Céréghino et al., 2001) and its use for bioassessment and conservation purposes is one of the main applications of

such approaches. In Britain, for example, invertebrate assemblages form the basis for the classification of unpolluted rivers and are used to develop procedures for predicting faunal assemblages at given sites from a small set of physicochemical variables (Wright et al., 1984). On the other hand, when these classification schemes are coupled with the description of type communities and/or indicator taxa, the potential value for understanding distribution patterns and conservation purposes becomes greater. Ecological indicators, that is species or groups of species that reflects the state of the environment of which it is an integral and valuable part (*sensu* McGeoch 2002) has been widely used in scientific studies during the last century and were first used scientifically for assessing water quality (McGeoch et al., 2002). There is clearly a need for the identification of characteristic or indicator species in the fields of nature monitoring, conservation, and management (Dufrene and Legendre, 1997).

The Mediterranean semi-arid regions are characterised by unevenly distributed water resources, high hydrological variability (low rainfall irregularly distributed in time and space), heterogeneous topography and anthropogenic pressure. The landscape in south-eastern Spain ranges from Mediterranean conifer forests in mountain ranges along major nature reserves in the NW, to arid and semi-arid shrublands further south-east. This longitudinal gradient in altitude and climate is coupled with a human density gradient. The river network has low populated forested headwaters, intermediately populated agricultural midlands with intense flow regulation, and densely populated cities in the lowlands.

Moreover, streams in arid regions often develop complex salinity gradients. The localized geological distribution of salt deposits and the fluctuations in discharge due to variable rainfall and groundwater inputs create a complex mosaic of salt concentrations (Magdych, 1984). Natural salinity gradients are well documented in the Segura River basin (Millan et

al., 1988; 1993; 1996; Vidal-Abarca et al., 1992; Moreno et al., 2001; Mellado et al., 2002). Some of the tributaries flow across salt-rich rocks such as sedimentary Miocene marls rich in sodium chloride and gypsum and therefore their salinity is naturally high (Moreno et al., 1997; 2001; Gómez et al., 2005). Saline streams have marked differences in biotic composition compared to their freshwater counterparts in the same region (Aboal 1989; Moreno et al., 1997; 2002).

Our objectives were: 1) identify classes of streams based on the macroinvertebrate assemblages; 2) provide consistent indicator taxa for the identified stream classes and 3) identify the main environmental variables (natural and anthropogenic origin) leading to the observed broad-scale macroinvertebrate distribution patterns. The general working hypothesis was that the spatial structure of the macroinvertebrate assemblages will reflect the primary environmental gradients observed across the Segura River basin.

2. Methods

2.1. Study area

The Segura River basin is located in the south east of the Iberian Peninsula (Figure 1). It flows from the NW to the SE and drains a basin of 14432 km^2. The climate ranges from humid in the mountains of the Northwest to semiarid elsewhere. The lithology of the plains is characterised by the predominance of limestone and Miocene and Keuper marls with some volcanic areas, whereas calcites and dolomites dominate the mountain headwaters. Quaternary deposits (clay, sand and gravel) form the river alluvia and cover rivers surrounding areas as a thin layer.
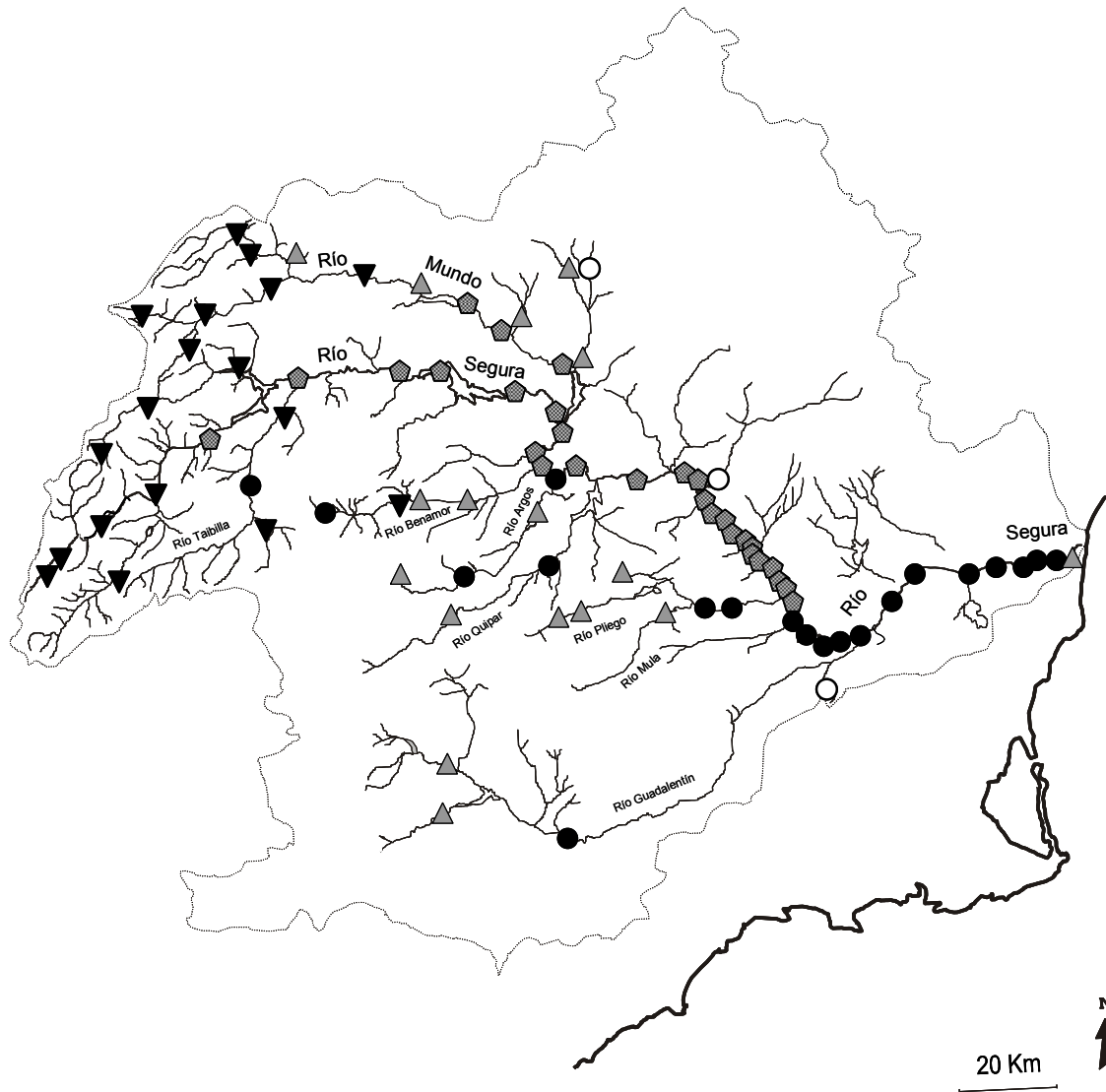
Figure 1. Study area showing the sampling sites. The different symbols used to plot sites correspond to the stream groups identified and discussed in the text: empty circles, group 1; black circles, group 2; shaded hexagons, group 3; Grey up-triangles, group 4 and black down triangles, group 5.

2.2. Sampling design

We sampled macroinvertebrates at 112 sites across a wide range of environmental conditions and water-quality in the Segura River basin (SE Spain). We used a spatially as well as temporally stratified sampling design to ensure adequate representation of stream types and environmental conditions. Nearly 20 tributaries were sampled. Some of streams (usually the smaller ones) were sampled at only one site per stream while larger tributaries

(considering catchment area or length) were sampled in 4 to 11 sites along their longitudinal gradient. We also took samples from the mainstem of the Segura River (48 sampling sites along the longitudinal axis). The surveys were undertaken over four seasons in 1987: winter (February 22 to March 19), spring (May 1 to June 4), summer (July 1 to August 8) and autumn (November 1 to December 10). The majority of sites were visited on all four occasions whereas remaining sites were sampled just once (5), twice (7) or three times (13). Only sites sampled in two or more surveys were considered in this study.

2.3. Macroinvertebrate sampling

Semiquantitative sampling was carried out by two operators by energetically kicking and disturbing substrate and vegetation and collecting all the suspended material with two square hand nets (900 cm^2 opening, 1 mm mesh). Wide and deep reaches were sampled only along the stream banks where it was feasible to sample with this method. At each site all meso- and microhabitats within ~ 100 meters stream reach were sampled and pooled together. This sampling protocol was designed to best characterize the diversity and relative taxonomic composition of the macroinvertebrate community. The entire sample was preserved in 70% ethanol and returned to the laboratory for subsequent sorting and identification. All organisms in a sample were sorted in white trays and identified to the lower taxonomic level that was achievable (usually genus or species). For data analysis we aggregated identifications to a genus level for consistency among faunal groups and also avoiding possible identification errors or taxonomic changes. Some groups were identified to higher taxonomic categories such as family (Diptera and certain Trichoptera), subfamily or tribe (Diptera: Chironomidae). Nematoda, Hydracarina and Oligochaeta were not identified further. In this study, which focuses on broad spatial patterns, we pooled the seasonal abundance data sets (by summing seasonal abundances for each site) into an

annual data set consisting of 112 sites X 176 taxa (see Appendix 1). We eliminated samples from the annual data set that had fewer than 100 organisms to avoid insufficient sampling noise. A final matrix with 87 sampling sites and 176 taxa was used for this study.

2.4. Environmental variables

A total of 28 environmental variables were measured or calculated for each site. Altitude (m.a.s.l.), latitude (degrees-N) and longitude (degrees-W) were calculated using 1:50,000 topographic maps. Water and air temperature, dissolved oxygen, pH and electric conductivity measurements were also taken in the field by standard portable equipment. Additionally, 2 litres of water were collected in each site and carried to the laboratory for suspended solids (mg/L, by filtration) and chemical analyses. Nutrient concentrations (phosphates, nitrates, nitrites and ammonia) were estimated by colorimetric methods. Total alkalinity was estimated by the volumetric method, Chemical Oxygen Demand (ChOD, mg/L) was measured by the dichromate method. Five-day biochemical oxygen demand (BOD$_5$) was calculated by dilution. Sulphates, sodium and potassium were estimated by ionic chromatography. Additionally, visual estimates of several categorical habitat variables were used: depth (1=very shallow; 2= shallow; 3= deep; 4= very deep), current velocity (0 = no flow; 1= very slow; 2= slow; 3= fast), substrate size (1=silt; 2= sand; 3= gravel; 4= pebble-cobble; 5= boulder-rock), substrate stability (0= immobile, 1: moderately mobile; 2=mobile), dominant organic matter fraction (1=Absent; 2=CPOM ; 3=FPOM; 4=UPOM), submerged macrophyte cover (0=absent; 1=sparse; 2=mid-cover; 3=dense-cover) and filamentous algae cover (0=absent; 1=sparse; 2=mid-cover; 3=dense-cover). Finally, climatic variables (mean annual precipitation, P and temperature, T) were obtained from the Phytosociological Research Center worldwide bioclimatic classification system database (available at http://www.globalbioclimatics.org). Each site was assigned data from the nearest climatic station and a total of 30 stations were selected across the catchment.

2.5. Data análisis

Stream sites classification was performed using fourth root-transformed abundance data and the corresponding Bray-Curtis similarity distances using a group-average clustering procedure (UPGMA). A total of 50 rare taxa (occurrence at <5% of sites) were removed from the data set because they usually create noise that obscures patterns in classification analysis (Gauch, 1982). After that, using the same similarity matrix, non-metric multi-dimensional scaling (MDS, Kruskal and Wish, 1978) was applied to visualize spatial patterns of community structure among the previously classified clusters. Site groups were enclosed by kernel density contours. MDS ordination attempts to place the samples in an arbitrary 2-dimensional space such that their relative distances apart match, in rank order, the corresponding pair-wise similarities: nearby samples have similar communities, and vice-versa. The stress value was recorded as a measure of the ordination effectiveness on preserving the similarity ranks. Site classification was also superimposed in the study area map (Figure 1) to show the geographic patterns arising from macroinvertebrate data.

To identify indicator taxa of the site groups, the Indicator-Value procedure was used (IndVal, Dufrêne and Legendre, 1997). This method identifies indicator taxa that best characterize groups of sites. The indicator value index (IV) reaches its maximum when all individuals of a species are found in a single group of sites and when the species occurs in all sites of that group. The statistical significance of the species indicator values is evaluated using a randomization procedure. This method identifies indicator species for typologies obtained by any hierarchical or non-hierarchical classification procedure and its use is independent of the classification method (Dufrêne and Legendre, 1997; McGeoch and Chown, 1998).

Finally, in order to establish the main links between environmental variables and patterns of multivariate community composition, we applied and compared the results of two different techniques, the BIOENV procedure (Clarke and Ainsworth, 1993) and canonical correspondence analysis (CCA, Ter Braak, 1986). BIOENV exhaustively searches for the combination of environmental variables that produces a similarity matrix that has the highest correlation with the biotic similarity matrix of sites (the same matrix obtained 4th root-transformed abundance data and Bray-Curtis distances). Environmental similarity matrices were calculated using normalized Euclidean distance and correlations were calculated using the weighted-Spearman rank correlation coefficient (ϱ_w, Clarke and Ainsworth, 1993). The environmental variables included in BIOENV analyses (Table 1) were log (x+1) transformed and standardized to validate the use of Euclidean distances in the environmental similarity matrices (Clarke and Warwick, 1994). The initial set of 36 environmental variables was reduced to 28 variables by identifying subsets of highly intercorrelated variables (r > 0.9) and excluding all but one of each subset prior to analysis. It was not computationally feasible to search the full space of $2^{28} + 1$ possible subsets of environmental variables, so the BVSTEP procedure was adopted as described by Clarke and Warwick (1998). This is a stepwise procedure analogous to stepwise multiple regression, in which subset selection proceeds incrementally. At each iterative stage, the single variable which maximally increases ϱ_w is added to the existing subset (formerly a random subset of variables), and there is also an elimination step in which the effect of dropping one variable at a time is considered. Forty random restarts that considered 50% of variables were run.

Canonical correspondence analysis was also performed using the same data sets as above. Environmental factors and abundances were log-transformed prior to analysis and rare species were down weighted. Two Monte Carlo permutation tests (1000 permutations) were used to assess the significance of the species-environment relationship of the first

	GROUP 1 (N=3)			GROUP 2 (N=20)			GROUP 3 (N=29)			GROUP 4 (N=17)			GROUP 5 (N=18)		
Group description	Ramblas			Lower Segura River polluted sites			Middle Segura/Mundo reaches			Main tributaries			Mountanious headwaters		
PHYSYCHO-CHEMICAL VARIABLES																
Water Temperature (Wt)	16.6	± 3.1	(13 − 18.5)	17.5	± 2.0	(14 − 21.5)	16.4	± 3.9	(13.25 − 35.25)	17.7	± 2.5	(14.975 − 23)	13.7	± 4.0	(7.375 − 23)
Air temperature (At)	23.5	± 5.3	(17.5 − 27.5)	21.1	± 2.9	(14 − 26)	22.1	± 2.8	(17.125 − 30.5)	23.4	± 2.3	(20.6 − 28.33)	19.3	± 4.5	(10.75 − 29)
pH	7.6	± 0.1	(7.48 − 7.72)	7.7	± 0.2	(7.33 − 8.14)	8.1	± 0.1	(7.90 − 8.39)	7.9	± 0.4	(6.38 − 8.28)	8.0	± 0.6	(6.2 − 8.33)
Dissolved Oxygen (Oxy)	9.4	± 0.3	(9.2 − 9.75)	7.2	± 2.9	(2.8 − 12.9)	10.7	± 0.5	(9.35 − 12.17)	10.4	± 1.4	(8.15 − 13.55)	11.6	± 0.8	(10.23 − 12.93)
Electric conductivity (E-con)	7057.8	± 5837.1	(2780 − 13707.5)	4556.7	± 4498.0	(604.33 − 18880)	1187.7	± 564.6	(406.75 − 2195.75)	2910.0	± 3435.8	(629.75 − 14682.5)	524.3	± 125.9	(321.75 − 751.75)
Suspended solids (TSS)	23.0	± 13.8	(7.2 − 33.15)	77.7	± 86.7	(21.37 − 401.57)	36.1	± 25.9	(7 − 140.08)	21.2	± 17.5	(5.4 − 73.03)	13.2	± 20.3	(1.83 − 70.98)
Chemical oxygen demand (ChOD)	13.9	± 19.4	(2.5 − 36.38)	54.6	± 88.4	(5.5 − 366.67)	4.4	± 1.9	(1 − 7.7)	7.3	± 9.9	(0 − 40.93)	1.7	± 1.1	(0.5 − 5.5)
Biological oxygen demand (DBO_5)	2.5	± 2.0	(0.43 − 4.43)	22.5	± 41.4	(2.33 − 148.7)	3.0	± 0.7	(1.5 − 5.03)	2.5	± 1.5	(0.17 − 6.65)	2.3	± 0.5	(1.07 − 2.85)
Nitrate (NO_3^-)	0.092	± 0.159	(0 − 0.28)	8.775	± 21.992	(0.17 − 100)	0.189	± 0.312	(0 − 1.55)	0.160	± 0.326	(0 − 1.25)	0.025	± 0.045	(0 − 0.15)
Phosphate (Phos)	0.042	± 0.072	(0 − 0.125)	11.157	± 21.730	(0 − 84.57)	0.158	± 0.294	(0 − 1.5)	0.161	± 0.312	(0 − 1.1)	0.025	± 0.075	(0 − 0.3)
Ammonia (NH_4^+)	18.989	± 19.779	(0.37 − 39.75)	21.568	± 23.214	(0 − 100)	4.718	± 3.175	(1.65 − 11.9)	13.340	± 9.929	(0.9 − 33.13)	2.090	± 1.219	(0.38 − 5.1)
Nitrite (NO_2^-)	0.033	± 0.058	(0 − 0.1)	5.754	± 22.194	(0 − 100)	0.042	± 0.064	(0 − 0.2)	0.124	± 0.311	(0 − 1.23)	0.004	± 0.013	(0 − 0.05)
Cloride (Cl^-)	1180.3	± 1510.4	(265.2 − 2923.6)	724.3	± 1058.2	(16.3 − 4289.3)	96.6	± 70.8	(11.2 − 230.53)	370.9	± 747.9	(26.6 − 3156.83)	15.4	± 7.6	(6.98 − 35.05)
Sulphite (Sulph)	1883.3	± 1028.1	(951.13 − 2985.98)	762.3	± 466.7	(81.5 − 1766.83)	270.5	± 166.6	(10.13 − 553.13)	717.9	± 767.6	(47.53 − 2435.93)	35.1	± 28.2	(3.7 − 87.8)
Sodium (Na^+)	645.3	± 762.8	(172.17 − 1525.33)	301.9	± 317.6	(4 − 1406.37)	64.7	± 57.4	(3.48 − 181.63)	219.6	± 419.2	(13.7 − 1773.88)	7.1	± 5.5	(2.03 − 22.45)
Potasium (K^+)	16.3	± 14.0	(8.1 − 32.43)	18.1	± 21.3	(1.5 − 100)	3.1	± 1.8	(0.6 − 7.1)	6.4	± 8.9	(1.28 − 38.25)	0.9	± 0.4	(0.4 − 1.95)
Alcalinity (Alka)	236.3	± 33.5	(203 − 270)	357.3	± 250.7	(187.33 − 1358.67)	180.0	± 23.9	(156.5 − 287)	241.5	± 62.8	(154.5 − 380.67)	208.9	± 39.1	(138.5 − 287)
GEO-CLIMATIC VARIABLES																
Latitude (North)	38.2	± 0.3	(37.906 − 38.561)	38.1	± 0.1	(37.661 − 38.247)	38.2	± 0.1	(38.051 − 38.51)	38.2	± 0.3	(37.71 − 38.585)	38.3	± 0.2	(38.082 − 38.621)
Longitude	1.4	± 0.2	(1.157 − 1.634)	1.3	± 0.5	(0.674 − 2.269)	1.6	± 0.3	(1.233 − 2.394)	1.7	± 0.3	(0.639 − 2.208)	2.4	± 0.2	(1.972 − 2.703)
Altitude (Alt)	376.7	± 211.3	(180 − 600)	204.6	± 318.1	(3 − 1260)	250.7	± 156.9	(60 − 620)	507.1	± 230.0	(0 − 900)	956.1	± 244.2	(620 − 1400)
Mean annual temperature (T)	16.3	± 1.2	(15.2 − 17.6)	17.1	± 1.6	(13 − 18.4)	16.9	± 0.9	(14.6 − 17.7)	16.1	± 1.0	(14.1 − 17.7)	13.3	± 2.1	(10.7 − 17.4)
Mean annual precipitation (P)	296.0	± 13.9	(280 − 305)	320.1	± 68.5	(271 − 555)	333.0	± 50.7	(280 − 445)	393.8	± 101.8	(271 − 550)	684.1	± 253.6	(330 − 1167)
HABITAT VARIABLES																
Valley width (Valley)	2.3	± 1.2	(1 − 3)	3.5	± 0.6	(2 − 4)	2.5	± 0.7	(1 − 4)	2.5	± 0.7	(1 − 4)	1.9	± 0.6	(1 − 3)
Depth	1.3	± 0.6	(1 − 2)	1.6	± 0.6	(1 − 3)	1.5	± 0.6	(1 − 3)	1.1	± 0.3	(1 − 2)	1.0	± 0.5	(0 − 2)
Width	1.3	± 0.6	(1 − 2)	1.8	± 0.6	(1 − 3)	1.9	± 0.3	(1 − 2)	1.6	± 0.5	(1 − 2)	1.4	± 0.7	(0 − 2)
Luminousity (Lum)	1.7	± 0.6	(1 − 2)	2.0	± 0.2	(1 − 2)	1.9	± 0.4	(1 − 2)	1.8	± 0.4	(1 − 2)	1.6	± 0.7	(0 − 2)
Current velocity (Current)	1.3	± 0.6	(1 − 2)	0.9	± 0.7	(0 − 2)	1.9	± 0.7	(1 − 3)	1.3	± 0.8	(0 − 3)	2.2	± 0.8	(0 − 3)
Substrate type (SubSiz)	3.3	± 2.1	(1 − 5)	0.5	± 0.8	(0 − 2)	2.0	± 1.1	(0 − 4)	1.2	± 1.1	(0 − 3)	2.8	± 0.4	(2 − 3)
Substrate movility (SubMov)	0.7	± 1.2	(0 − 2)	2.0	± 0.0	(2 − 2)	1.7	± 0.5	(0 − 2)	1.8	± 0.4	(1 − 2)	1.1	± 0.5	(0 − 2)
POM type (POM)	2.0	± 1.7	(0 − 3)	2.6	± 1.0	(0 − 3)	1.3	± 1.4	(0 − 3)	0.9	± 1.4	(0 − 3)	0.9	± 1.3	(0 − 3)
Submerged macrophyte cover (SubMac)	2.0	± 1.7	(0 − 3)	0.8	± 1.4	(0 − 4)	0.2	± 0.6	(0 − 3)	1.8	± 1.6	(0 − 5)	1.4	± 1.1	(0 − 4)
Bryophite cover (Bry)	0.3	± 0.6	(0 − 1)	0.1	± 0.2	(0 − 1)	0.0	± 0.2	(0 − 1)	0.2	± 0.4	(0 − 1)	0.7	± 0.8	(0 − 2)
Filamentous algae cover (FilAlg)	1.7	± 1.5	(0 − 3)	0.5	± 1.1	(0 − 4)	0.6	± 0.9	(0 − 4)	0.6	± 0.8	(0 − 3)	1.7	± 1.2	(0 − 4)
COMMUNITY VARIABLES																
Number of taxa (S)	21.7	± 4.0	(17 − 24)	14.0	± 5.9	(4 − 27)	17.8	± 5.3	(8 − 27)	35.9	± 8.5	(23 − 54)	52.9	± 13.1	(33 − 78)
Pielou evenness (J´)	0.4	± 0.2	(0.24 − 0.61)	0.5	± 0.2	(0.09 − 0.82)	0.6	± 0.2	(0.04 − 0.77)	0.5	± 0.2	(0.16 − 0.77)	0.6	± 0.1	(0.34 − 0.77)
Shannon diversity (H´)	1.2	± 0.7	(0.68 − 1.95)	1.2	± 0.5	(0.14 − 2.15)	1.6	± 0.5	(0.13 − 2.33)	1.9	± 0.6	(0.54 − 2.70)	2.4	± 0.6	(1.37 − 3.24)

Table 1. (previous page) Averaged physicho-chemical, geo-climatic, habitat variables, Richness (S), Pielou evenness (J') and Shannon diversity (H') (mean ± SD and range) for each of the UPGMA classification groups. (Number of sites N from each group in brackets)

canonical axis and of all canonical axes together. Two CCA plots were constructed, one showing sites (with UPGMA clusters superimposed) and environmental variables vectors and another showing the macroinvertebrate taxa ordination.

3. Results

3.1. Classification of sites

UPGMA classification using Bray-Curtis similarity distances discriminated five groups of sites at approximately 32% similarity (Figure 2). The first separated cluster, Group 1, was composed of three small tributaries. The second cluster, Group 2, was composed of 20 sites, of which 12 were located in the lower reaches of the Segura River, in or close to the main cities in the region, and the remaining 8 were sites from different tributaries downstream of relatively important urban centres (Figure 1). Group 3 was the biggest cluster and consisted of 29 sites, 24 of which were located along the middle course of the Segura River, 4 were also middle reaches of the Rio Mundo (the main tributary in the Rio Segura) and 1 was part of the Benamor stream, close to its confluence with the Rio Segura (Figure 1). 19 out of 24 sites in Group 3 were situated close to or even inside small urban areas, 4 sites were immediately downstream of reservoirs and 3 were just after the confluence of a tributary in the main Rio Segura. 17 sites made up the next discriminated cluster, Group 4 A total of 15 sites distributed across 12 streams, mainly located across the middle part of the river network, formed the bulk of this group (the remaining two sites

were in the middle Rio Mundo, and the mouth of the Rio Segura) Finally, Group 5 was composed of 18 sites in the headwaters of the Segura and Mundo streams.

Geographic patterns appeared evident when the classified groups were plotted in the map (Figure 1), with Group 5 sites located in the NW mountains, Group 2 located in the lower Segura River mainstem in the SE, Group 3 sites situated along the middle courses of the Segura and Mundo rivers at intermediate longitudes and Group 4 sites located also at intermediate longitudes, mainly in the southern basin, where a number of tributaries run through agricultural areas.

3.2. Ordination of sites. Description of stream group characteristics

NMDS ordination plot of sites using fourth root-transformed abundance data and Bray Curtis similarity distances effectively discriminate our five UPGMA groups (Figure 3). The 2-d best solution (using 100 random start configurations) provided an acceptable stress value of 0.18. Kernel density contours (90% probability) around the groups showed little overlap. Group 5 sites were plotted on the left side while Group 2 sites were located near the upper-right corner. Groups 1, 3 and 4 showed intermediate positions along the first axis, but were well separated along the second NMDS dimension, with Group 3 sites on the lower half and Group 4 and more clearly Group 1 occupying the upper positions.

Group 1, composed of 3 semi-arid stream sites was characterized by high electric conductivities (with high chloride, sulphate and sodium concentrations), low nutrient loads and biological oxygen demand and relatively high ammonia concentrations. They were narrow, open and shallow channels some having stable hard-bottom substrates, intermediate submerged macrophyte and filamentous algae cover and presenting low

diversity and evenness values. Nevertheless, broad environmental differences existed between these three sites (See Table 1 for a detailed description of physicho-chemical, climatic, community and habitat variables in each cluster).

Group 2 was characterized by the lowest water quality values (Table 1). Climatic variables were typical of arid or semi-arid environments, with the highest mean annual temperatures and potential evapotranspiration among the study sites and annual rainfall values close to 300 mm. They were wide and deep open channels located in wide valleys. Current velocity was very slow. Substrate was highly instable and silt-dominated while UPOM was the main sediment organic fraction. Aquatic vegetation cover was low. Macroinvertebrate communities were characterized by the lowest richness and diversity values.

Group 3 sites were characterized by semi-arid climate, low altitude, relatively high water quality and low mineralization. Dominant substrate was loose gravel and CPOM was the main organic sediment fraction. They were wide and deep reaches located in wide valleys with open canopies. Current velocity was moderate. Aquatic vegetation was almost inexistent while riparian corridors were well structured. Macroinvertebrate communities showed low taxa richness and intermediate evenness and diversity values.

Group 4. The main characteristics of this group were: low orders, middle altitudes, intermediate aridity; moderate water quality (good oxygenation, moderate conductivities and mineralization, relatively low nutrient loads, suspended solids and BOD_5, and moderate ammonia and nitrite contents). Habitat was characterized by relatively wide and shallow channels with low to moderate current velocity. Substrate was sandy and CPOM was the main organic fraction. Submerged macrophytes were moderately abundant. Taxa richness and Shannon diversity values were high while Pielou evenness showed intermediate values.
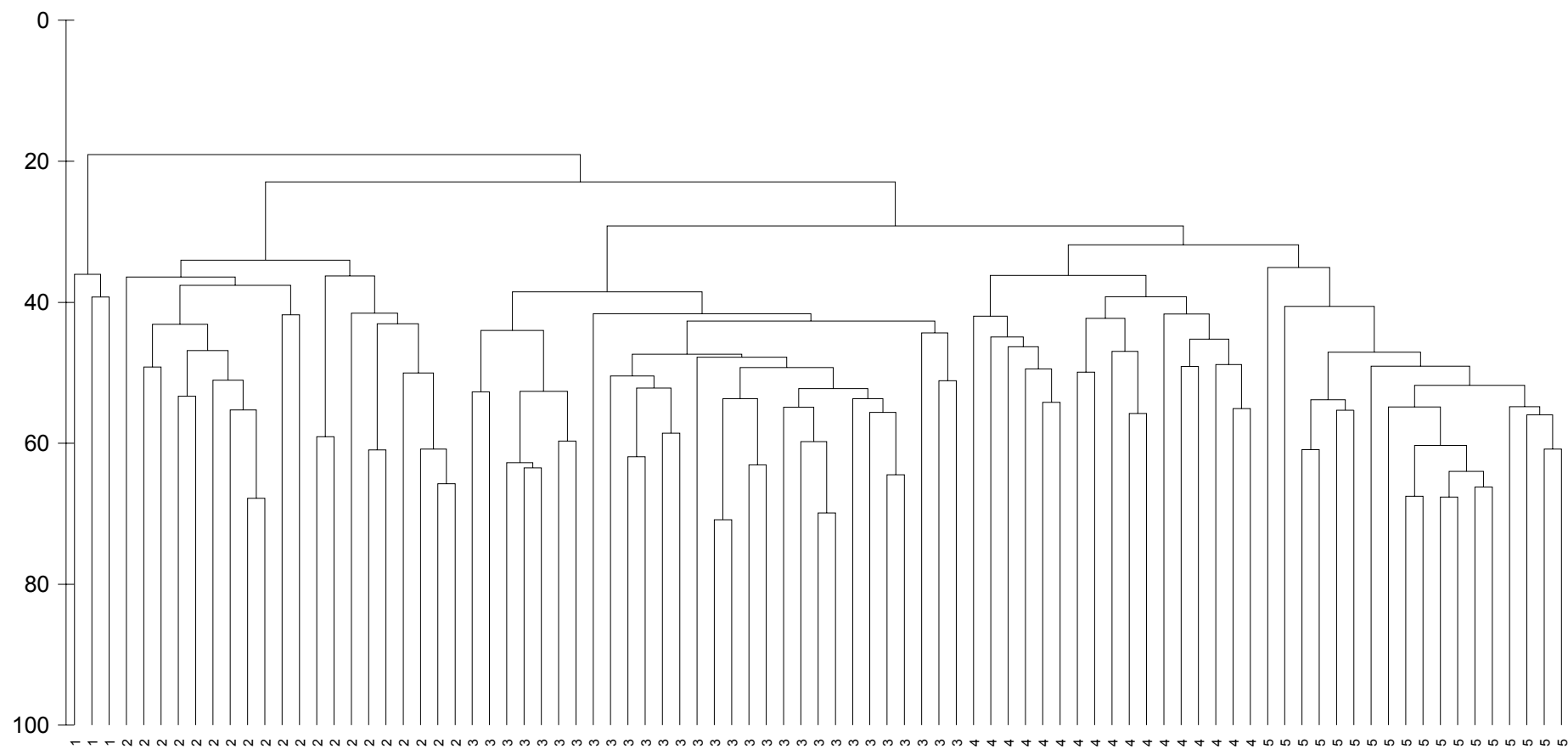
Figure 2. Group average clustering (UPGMA) dendrogram of the study sites based on their Bray-Curtis similarities. Sites are labelled with numbers (1 to 5) identifying stream groups.
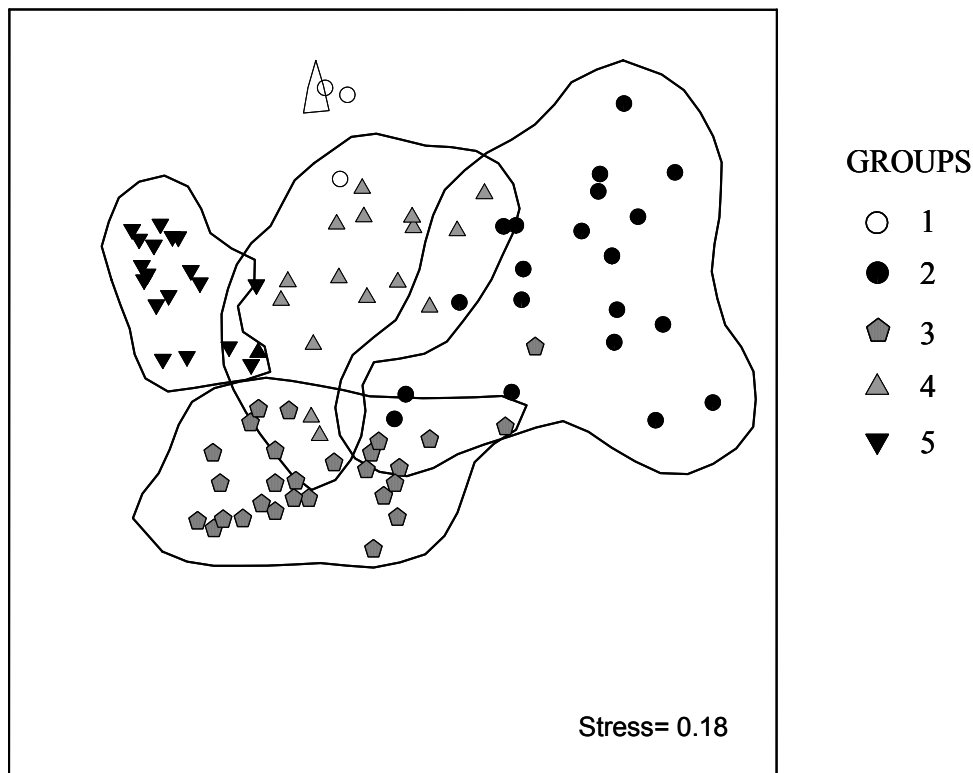
Figure 3. Non-metric Multiple Dimensional Scaling (MDS) ordination plot of the study sites based on their Bray-Curtis similarities. Symbols identify the stream groups defined by UPGMA (see legend). Kernel density contours (90%) were plotted for each group of sites.

Group 5 sites were located in high altitude mountainous conifer-forested areas inside or very near the Sierra de Cazorla, Segura and Las Villas nature reserve, in the more humid NW part of the basin (Figure 1). Climate was characterized by relatively low mean annual temperature and high annual rainfall (close to 700 mm). Water quality variables reached their best relative values in this group, with very low values (relative to our natural regional standards) of mineralization, nutrient contents, suspended solids and BOD_5, nitrite and ammonia. Moderately narrow and shallow stretches located in V-shaped valleys formed this group of sites. Cobbles and boulders were the main substrate types and CPOM dominated the organic fraction. Submerged macrophytes were scarce while filamentous algae cover was moderate. There appeared some bryophytes as well. Riparian vegetation was dominated by trees. Mean taxa richness (with 17 taxa more than group 4, 35 more than

group 3 and 39 more than group 2), diversity and evenness reached their peak values in these sites.

3.3. Environmental variables explaining community patterns

The BVSTEP procedure identified a 5 variables subset giving the highest correlation (ϱ_w = 0.478, see Table 2). However, a second subset identified by this technique that gave a similar correlation (ϱ_w = 0.475) included only 3 variables (dissolved oxygen, electric conductivity and altitude) and was therefore considered the most useful model. Moreover, these three variables were present in all 6 subsets identified. Adding two more variables, nitrate and annual rainfall, correlation was minimally improved, while when 7, 10 or 12 variables were selected, correlations decreased. Variables included in each model are listed

Table 2. Six best subsets results found by BVSTEP on 28 environmental variables explaining macroinvertebrate patterns. Correlation coefficient for each subset (ϱw) is indicated. Variable codes can be found in Table 1.

	Subconjuntos de variables					
Nº de variables	**5**	**3**	**12**	**10**	**7**	**3**
	Oxy	Oxy	Stemp	Stemp	Oxy	E-con
	E-con	E-con	Oxy	Oxy	E-con	ChOD
	NO_3^-	Alt	E-con	E-con	Alk	Alt
	Alt		NO_3^-	NO_3^-	Width	
	P		Alk	Alk	SubSiz	
			Valley	SubSiz	Alt	
			Current	SubMac	P	
			SubSiz	FilAlg		
			SubMac	Alt		
			FilAlg	P		
			Alt			
			P			
Correlación (ρ_w)	0.478	0.475	0.473	0.473	0.466	0.459

in Table 2. Geo-climatic variables (altitude, air temperature and mean annual precipitation) and water quality (electric conductivity, dissolved oxygen and nitrate) were the most important factors explaining macroinvertebrate patterns. Among the habitat variables (present only in the larger subsets), channel width, valley width, substrate size, current velocity, submerged macrophyte and filamentous algae were selected.

Canonical correspondence analysis ordination plot was fairly similar to the MDS one, with the classified site groups occupying approximately the same relative positions. Nevertheless, two extreme outliers were identified in the CCA plot: Site 61, located near Group 5 sites, was classified as a polluted site (Group 2) according to the community because it is located in a reservoir, and Site 53, the mouth of the Segura River, biologically classified as a Group 4 site but now positioned as a polluted site due to environmental constraints apparently not affecting the community.

The environmental variables used in the analysis are represented in the biplot (Figure 4) by arrows, which point in the direction of maximum change in the value of the associated variable. The arrowhead co-ordinates on each axis are essentially the correlations of the environmental variable with that ordination axis. The length of the arrow is therefore proportional to the maximum rate of change for the variable, so those variables with relatively short arrows do not vary much across the ordination plot and therefore were not represented for clarity in Figure 4.

The two first CCA axes explained 36.3% of the species-environment relationship. The Monte Carlo tests (999 permutations) showed that axis 1 and all the axes were statistically significant ($p \approx 0.001$). Interset correlations of environmental variables with CCA environmental axes were as follow: Axis 1, that accounted for 24.8 % of variance in of the

biota-environment relationship was positively correlated to sulphate (0.87), electric conductivity (0.83), mean annual temperature (0.75), nitrate (0.75), chemical oxygen demand (0.70), total suspended solids (0.69), valley width (0.64) and water temperature (0.63) and negatively with mean annual rainfall (-0.78), altitude (-0.75), pH (-0.72), substrate size (-0.71), dissolved oxygen (-0.65) and current velocity (-0.58). The second axis, that accounted for 11.5% of the biota-environment relationship, showed its highest positive correlations with submerged macrophyte cover (0.53) and alkalinity (0.38) and negative with mean annual temperature (-0.38), channel width (-0.36) and depth (-0.27). The eigenvalues for the CCA axis 1 (0.328) and 2 (0.152) were similar to those for a CA (0.358, 0.210), indicating that these environmental variables were important in explaining the macroinvertebrate variance. Eigenvalues greater than 0.3 indicate a very strong gradient along the corresponding axis (ter Braak 1986).

Figure 5 illustrates the position of the invertebrate assemblages along the same gradients: along the first axis, one of the extreme positions, that correspond to Group 2 polluted sites is occupied by *Helochares lividus*, Ephydridae, *Chironomus gr.thummi/plumosus*, *Asellus aquaticus*, *Physella acuta* or Oligochaeta, which represent the most tolerant taxa, whereas stoneflies as *Leuctra* spp, *Capnioneura* spp or *Isoperla* spp, mayflies as *Habrophlebia* spp, *Epeorus torrentinum* or *Centroptilum luteolum* and caddisflies as Brachicentridae or Psychomyidae characterize the less impacted habitats on the left side, where Group 5 sites were plotted. Concerning the second CCA axis, *Oligoneuropsis skhounate, Ecdyonurus* spp*, Potamanthus luteus, Procloeon* spp*, Cheumatopsyche* spp or *Gomphus* spp and the crustacean *Atyaephyra desmarestii*, are positioned in the negative extreme, where sites from wide and relatively deep reaches from middle courses (Group 3) were located, while some other are in the upper extreme: *Hydrobiidae NID, Orthetrum* spp*, Plea minutissima, Ferrisia* spp, *Naucoris maculatus, Sigara* spp, *Velia* spp, *Ochthebius* spp, *Enallagma* spp, *Coenagrion* spp or *Echinogammarus* spp.
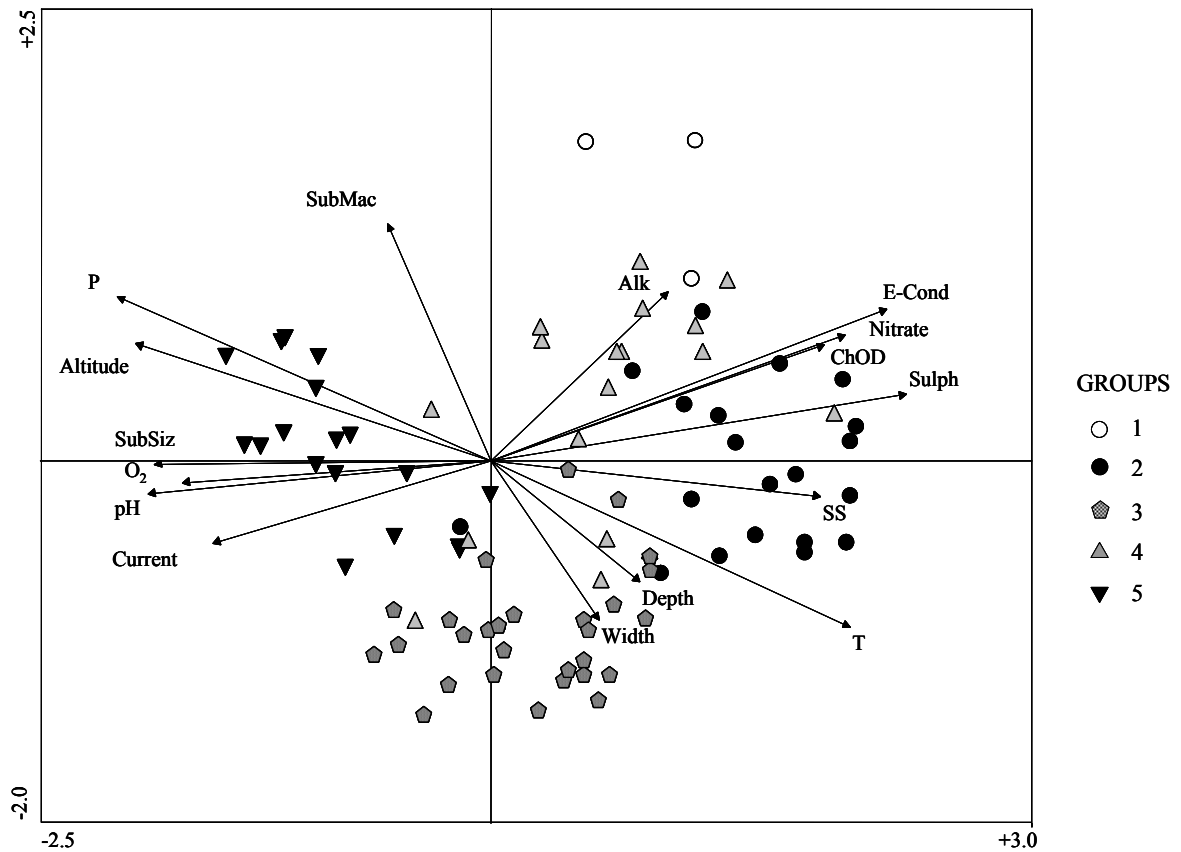
Figure 4. Canonical correspondence analysis (CCA) ordination biplot showing study sites and environmental variables vectors (shorter vectors were eliminated for clarity). Again, site symbols identify the stream groups defined by UPGMA (see legend).

They occupied relative positions in the CCA that corresponded to groups 1 and 4.

3.4. Indicator taxa

The IndVal method using our 126 taxa x 87 sites data set and 9999 permutations found some good indicators for the macroinvertebrate-based stream typology defined by UPGMA classification (Figure 6). In general, among the identified indicators, Coleoptera was the dominant order with twelve taxa, followed by Diptera and Ephemeroptera – seven taxa each –, Trichoptera – six taxa –, Plecoptera and Odonata – five taxa each –,
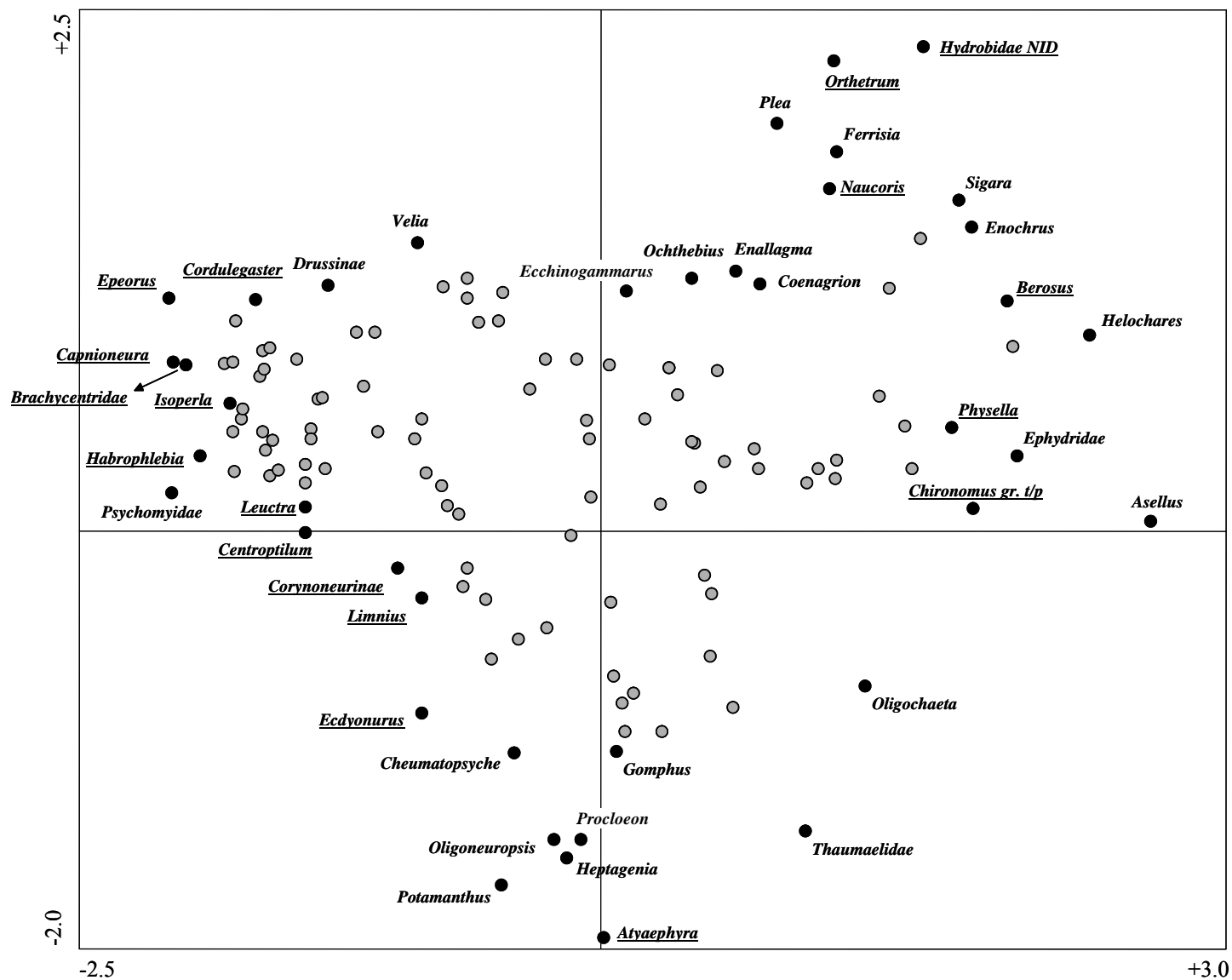
Figure 5. Canonical correspondence analysis (CCA) ordination plot of taxa. Only those taxa located at the limits of the cloud (black circles) were labelled for clarity. Underlined taxa were identified as significant indicators by IndVal

Mollusca and Heteroptera – four taxa – and Decapoda or Tricladida both with one indicator.

At the second level, where the three sites forming Group 1 were separated, IndVal identified 10 indicator taxa for this group. Some of them were very good examples of characteristic taxa (significant IV around 90%). as Hydrobiidae (unknown genera), *Naucoris maculatus*, *Hydrometra stagnorum* or *Orthetrum* spp. Only one taxon, the midge subfamily Orthocladiinae (IV=99.7), was found to be a significant indicator of the remaining sites at the same division level. At the third cluster division, where heavily polluted sites forming Group 2 were split up, only one significant indicator was found for this cluster, the midge species group *Chironomus gr. thummi/plumosus*. Two common mayfly taxa, *Caenis* spp and *Baetis* spp and the caddisfly *Hydropsyche* spp were indicative of the remaining sites. The fourth cluster division separated the Group 3 sites, with the crustacean *Atyaephyra desmarestii* as their only one indicator taxa. Group 4 and Group 5 were characterized by Simuliidae, *Haliplus* spp and *Nepa cinerea*. Finally, Group 4 characteristic taxa showed relatively low indicator values, ranging from 30.5 to 44.4. Group 5 was the cluster with the bigger indicator taxa set (up to 39 significant indicators, with IV ranging from 31.8 to 92.8). It comprised up to ten coleopterans, seven ephemeropterans, six trichopterans, six dipterans, five plecopterans, four odonatans and one triclad (Figure 6).

4. Discussion

Results are in accord with previous works from the basin where the main trends showed by aquatic biota were dominated by factors such as electric conductivity and altitude, linked to a NW-SE geographic and climatic gradient.

Figure 6. UPGMA classification dendrogram showing the significant indicator taxa with their indicator value (in brackets) calculated by the INDVAL program for each of the cluster divisions.
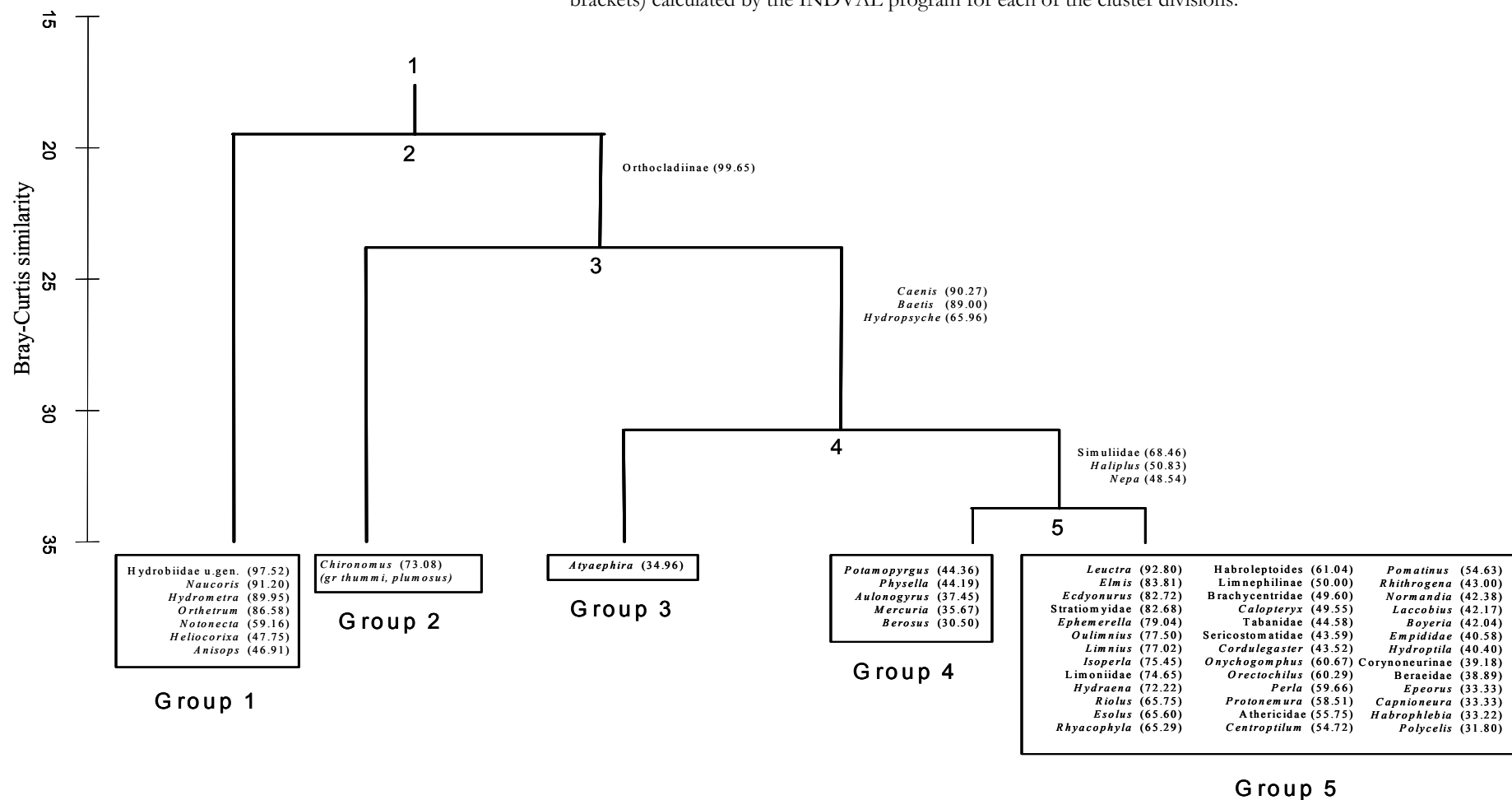
Macroinvertebrate communities from the Segura River basin are still poorly known. Despite some local government reports or sparse faunistic references on certain taxa (Millan et al., 1988; 1993; Gil et al., 1990; Ubero-Pascal al., 1998; Bonada et al., 2004), little information about general distribution, environmental factors or any other ecological aspect of these stream assemblages has been published to the date (but see Mellado et al., 2002; Vivas et al., 2004). We present important baseline information about the environmental variables affecting the distribution patterns of macroinvertebrate communities in our region. Moreover, a long-term information point (we treated 1987 data) has been established for comparative studies in the future.

4.1. Site classification and ordination. Indicator taxa

Freshwater conservation strategies and monitoring programmes typically rely on classification of sites based on a single biotic group, most typically benthic macroinvertebrates. Our site classification (and a posteriori ordination) resulted in four main distinguishable classes. Physicho-chemical, geo-climatic, habitat and community variables allowed us to basically describe each group. Three of the groups were each positioned in a distinct portion of the longitudinal gradient of the Segura and Mundo rivers (Groups 5 –headwaters-, 3 –middle courses- and 2 –lower courses-, Figure 1). Group 4 was composed of tributaries flowing into middle courses of the main axis. Mellado et al. (2002) found a very similar classification using family level presence-absence data across sixty sites in 1998, although one more group ("Group 5" in Mellado et al., 2002) was defined, mainly composed of small saline streams that were not sampled in 1987 (this study). Two of the three sites from Group 1 here were part of that group in 1998.

Faunal composition and community structure was markedly distinct among the classified groups, as the indicator value method (IndVal) and the CCA species plot showed. The IndVal method (Dufrêne and Legendre, 1997) enabled the identification of indicator taxa for each of the 5 groups and cluster hierarchy levels. Coleoptera was the most represented group. The indicator value of aquatic beetles has been pointed out in numerous works (Ribera and Foster, 1993; Ribera, 2000; Sánchez-Fernández et al., 2004). The group that showed most of the indicator taxa was Group 5, with almost 40 taxa. They were mostly good water quality indicators, stenotypic species typical of high altitude ranges and relatively low temperature tolerance such as the majority of EPT taxa, elmid beetles and some dipterans and odonatans. Stratiomyidae, a dipteran family usually known as a tolerant group (Alba-Tercedor and Sánchez Ortega, 1988; Chessman, 1995) was found as a very good indicator (IV=82.7%) for these streams, what we could justify because of the abundance of the genus *Oxycera* in semi-pristine streams from the upper catchment.

Group 4 indicators showed lower IVs. They were more eurytopic taxa, but were more abundant in these systems. There were three aquatic snails, *Physella acuta* and *Potamopyrgus antipodarum*, usually typical of stagnant waters or low flow streams and with very wide geographical distributions and *Mercuria emiliana*, an interesting hydrobid snail that is common in mineralized streams. Two water beetles: *Berosus spp*, with most of species with marked preferences for middle altitudes, shallow or lentic habitats and moderate to high mineralization (Sánchez-Meca et al., 1992), and *Aulonogyrus striatus*, the most eurythermic gyrinid species within the Segura River basin (Millan et al., 1996).

The decapod *Atyaephyra desmarestii* was found as an indicator of the middle courses of Group 3. This organism has shown preference for relative good water quality and a variety

of habitats, including reservoirs and channelizated reaches or rice fields (Micha et al., 1985; Rinderhagen et al., 2000; Fidalgo and Gerhardt, 2003).

The well known pollution tolerant taxa *Chironomus gr. thummi/plumosus* was the only indicator taxa for polluted lower reaches of Group 2. This species group has long been reported in numerous studies as one of the most pollution tolerant taxa (e.g. Hilsenhoff, 1987; Zamora-Muñoz and Alba-Tercedor, 1996; Walsh et al., 2001; Janssens de Bisthoven and Gerhardt, 2003).

Group 1 sites were characterized by relatively high IVs for some Heteroptera (*Naucoris maculatus*, *Hydrometra stagnorum*, *Notonecta* spp, *Heliocorixa vermiculata* and *Anisops* spp), one artificial taxa designated as Hydrobiidae u.gen. (too small to identify) and *Orthetrum* spp. Their position in the CCA (on the right upper corner) indicated preference for shallow streams with low flow, high macrophyte cover and high mineralization and nitrate contents. Nevertheless, with only three sites within this group in this study we have limited data in characterizing species-habitat relationships.Only groups 1, 2 and 5 presented values over 70%, a threshold showed by McGeoch et al. (2002) to remain consistent among different studies. It is important to note that a verification study (with new data collection in 1998) of the indicator taxa found in this work will be carried out to achieve a higher confidence (*sensu* McGeoch et al. 2002).

4.2 Environmental constraints

Understanding community patterns is important to manage target ecosystems. Invertebrate community structure reflects the influence of many variables, and discrete influences are difficult to identify. We used classification and ordination to examine multivariate

relationships among aquatic invertebrate community structure and abiotic variables. We identified electric conductivity, altitude and mean annual precipitation as the variables causing major differences in community structure along a broad study area characterized by marked contrasts in climatic and landscape features, as well as in anthropogenic pressure. Nutrient enrichment and dissolved oxygen were also variables exerting influence on macroinvertebrate spatial distribution patterns. In our work, electric conductivity showed very high correlation with chloride (r=0.98) and sulphate (r=0.88), so our results clearly support a principal environmental gradient linked to salinity. Some other variables related to eutrophy and organic pollution as nitrate, chemical oxygen demand or total suspended solids also pointed to the same direction of this gradient. So, salinity and pollution appeared as the keystone factors governing broad-scale macroinvertebrate distribution patterns in the Segura River basin. This main gradient was inversely correlated with the catchment longitudinal gradient, from freshwater, pristine, high altitude, humid headwaters in the NW to polluted lower reaches towards the more arid SE. A clear longitudinal gradient (from headwaters to lower reaches) in current velocity and substrate size discriminating our community types was also evident from CCA results.

The other important trend observed was related to stream size, depth and submerged macrophyte patterns: MDS and CCA ordinations separated along their second axes two groups of sites: on one hand, small sized shallow tributaries (Group 4), and on the other hand, wide and deep reaches from middle courses (Group 3). Severe reductions in water flow that result from water removal for irrigation and municipal supplies (a problem on many dry-land river systems, Davies et al., 1994; Vidal-Abarca et al., 2004) are the major human related impacts on southern Iberian rivers (Aguiar et al., 2002). Group 4 are small sized streams affected by water diversions as well as by nutrient enrichment from their agricultural watersheds. The fact that submerged macrophyte cover was negatively

correlated with stream width and depth can be explained as middle course sites from Group 3 usually are affected by flow regulation and channelization, both factors preventing the normal develop of aquatic plants (Armitage and Pardo, 1995; Wood et al., 1999).

Results indicate that the most influential variables for community structure were variables that usually operate at a regional scale, affected by climatic and/or geological patterns (mineralization, altitude, rainfall) or land use activities (eg. Nitrate). Some local physical habitat attributes as channel width, substrate size or current velocity (also governed by catchment scale features as geology, altitude or slope) were also important factors although to a lesser extent. Our findings suggest that the broad scale patterns observed in macroinvertebrate assemblages are not determined solely by local mechanisms acting within assemblages, but mainly result from processes operating at larger spatial scales. The integration of different spatial scales may be the key to increasing our understanding of the complex interacting factors that determine community composition.

On the other hand, variables such as altitude, geology and land use practices are highly intercorrelated. Another confounding effect could be the fact that habitat quality in our lowland urban watersheds is usually poor due to several interacting factors such as sedimentation from construction works, channelization, damming, bank erosion, loss of riparian habitats, etc. All of these have negative consequences for biological communities. Moreover, increasing conductivity and salinity from land use practices potentially confound the effect of natural longitudinal and salinity gradients. This make difficult to accurately assess the effect of one single variable on a broad complex catchment. In addition, many of these factors began affecting streams at the onset of human settlement, leading one to conclude that current ecosystem health may reflect centuries of pollution, degradation, and in some cases recovery (Nedeau et al. 2003). Despite these potentially confounding effects,

clear differences were nevertheless detected between communities under different environmental scenarios. Properly designed experiments are required to disentangle the array of confounding factors that influence community patterns in the Segura river basin.

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Appendix 1

Taxonomic unit	Taxa		Taxonomic unit	Taxa		Taxonomic unit (Code)	Taxa		Taxonomic unit (Code)	Taxa		Taxonomic unit (Code)	Taxa
Coleoptera	*Aulonogyrus*		**Heteroptera**	*Hydrometra*		**Trichoptera**	Hydropsyche		**Diptera**	Tipulidae		**Mollusca**	*Ancylus*
	Gyrinus			*Velia*			Cheumatopsyche			Limoniidae			*Ferrisia*
	Orectochilus			*Microvelia*			Sericostomatidae			Psychodidae			Hydrobiidae
	Peltodytes			*Aquarius*			Rhyacophyla			Dixidae			*Mercuria*
	Haliplus			*Gerris*			Psychomyidae			*Chaoborus*			*Potamopyrgus*
	Hydroglyphus			*Micronecta*			Brachycentridae			Culicidae			*Pseudamnicola*
	Graptodytes			*Cymatia*			Leptoceridae			Simuliidae			*Lymnaea*
	Laccophylus			*Heliocorixa*			Beraeidae			Tanypodinae			*Physella*
	Agabus			*Sigara*			Hydroptila			Tanytarsini			*Planorbis*
	Ilybius			*Corixa*			Agraylea			Chironomini			*Gyraulus*
	Hydaticus			*Aphelocheirus*			Limnephilinae			Chironomus gr. Thummi/riparius			*Melanopsis*
	Yola			*Nepa*			Drussinae			Orthocladiinae			*Theodoxus*
	Hygrotus			*Anisops*			Philopotamidae			Thaumaeliidae			*Pisidium*
	Hydrophilus			*Notonecta*			Glossosomatidae			Ceratopogonidae		**Crustacea**	*Asellus*
	Stictonectes			*Mesovelia*			Polycentropodidae			Stratiomyidae			*Echinogammarus*
	Deronectes			*Hebrus*						Empididae			*Atyaephyra*
	Nebrioporus			*Naucoris*						Tabanidae			*Austropotamobius*
	Oreodytes			*Plea*						Atherycidae			
	Rhantus		**Plecoptera**	*Nemoura*						Ephydridae			
	Meladema			*Protonemura*						Corynoneurinae			
	Dytiscus			*Amphinemura*						Anthomyidae			
	Cybister			*Thyrrenoleuctra*						Psychopteridae			
	Laccobius			*Leuctra*						Syrphidae			
	Helochares			*Capnioneura*						Dolichopodidae			
	Enochrus			*Capnia*					**Odonata**	*Calopteryx*			
	Hydphidrus			*Perla*						*Platycnemis*			
	Berosus			*Eoperla*						*Pyrrhosoma*			
	Anacaena			*Dinochras*						*Ischnura*			
	Coelostoma			*Isoperla*						*Cercion*			
	Hydraena			*Isogenus*						*Coenagrion*			
	Ochthebius			*Chloroperla*						*Enallagma*			
	Limnebius		**Ephemeroptera**	*Baetis*						*Onychogomphus*			
	Pomatinus			*Cloeon*						*Gomphus*			
	Dryops			*Procloeon*						*Boyeria*			
	Potamophilus			*Centroptilum*						*Aeschna*			
	Elmis			*Epeorus*						*Anax*			
	Esolus			*Rhithrogena*						*Cordulia*			
	Oulimnius			*Ecdyonurus*						*Oxygastra*			
	Limnius			*Heptagenia*						*Crocothemis*			
	Riolus			*Torleya*						*Sympetrum*			
	Normandia			*Ephemerella*						*Orthetrum*			
	Hydrochara			*Caenis*						*Libellula*			
	Elodes			*Paraleptophlebia*					**Other groups**	Tricladida			
	Helophorus			*Habroleptoides*						Oligochaeta			
	Noterus			*Habrophlebia*						Nematoda			
				Choroterpes						Hydracarina			
				Ephemera									
				Ephoron									
				Potamanthus									
				Oligoneura									
				Oligoneuropsis									

Chapter 3

Biological and ecological traits of stream macroinvertebrates from a semi-arid catchment. Patterns along complex environmental gradients

Chapter 3. Biological and ecological traits of stream macroinvertebrates from a semi-arid catchment. Patterns along complex environmental gradients

Abstract

The relationships between biological and ecological traits of macroinvertebrate communities and environmental attributes measured at different spatial scales was investigated in various streams with contrasting physical, chemical or landscape level attributes. We used a newly developed ordination technique, the RLQ analysis (Dolèdec 1996), that links an environmental table (R) with a faunal attributes table (Q) through a faunal abundance table (L) to provide insights into the relationships between habitat attributes and biological or ecological traits. We specifically address the following questions: Are there differences in the suites of biological and ecological traits from streams of contrasting environmental features?; Which are the principal traits involved in those possible trends?; Is there a match between certain ecological preferences and some corresponding life-history traits?; Which are the main environmental factors driving those differences and at which spatial scales are acting?; Are those differences in concordance with the River Habitat Templet and other relevant ecological theories?.

A major environmental axis explaining the distribution of communities and their distinctive biological/ecological features was obtained. This axis included both variables of anthropogenic pressure (agricultural and urban uses) and natural variability (climatic and geologic) that are strongly intercorrelated in the study area, with a clear spatial component.

We generally found typical attributes of species from frequently disturbed systems (small size, polivoltinism, dormancy, ovoviviparity, etc.) in the semi-arid SE part of the study area, while traits commonly occurring in more stable and favourable environments (big sizes,

merovoltinism, isolated eggs, etc.) were found in mountain forested catchments sites from the humid NW part.

The natural climatic variation was proposed as a *disturbance* axis of a theoretical habitat templet (driven by the more intense disturbance regime typical of semi-arid streams), while the anthropogenic pressure (mainly driven by intensive agriculture) summed to the natural increase in salinity due to differential geology was proposed as an *adversity* axis. Thus, our principal gradient was supposed as the diagonal of the templet, from the less disturbed freshwater streams in forested catchments of the humid north-west, to the most disturbed and often saline streams.

KEYWORDS: Biological traits, Ecological traits, semi-arid stream, macroinvertebrates, SE Spain, multivariate methods

1. Introduction

The distribution and abundance of stream macroinvertebrates are influenced by a variety of physical and biological factors. Abiotic factors, in particular those related to disturbance (Resh et al., 1988; Poff, 1992) and habitat heterogeneity, which provide shelter from disturbance (Sedell et al., 1990; Scarsbrook & Townsend, 1993; Minshall & Robinson, 1998) clearly determine the composition of invertebrate communities. Abiotic factors that influence community structure differ from system to system and with the spatial scale (Tate & Heiny, 1995; Vinson & Hawkins, 1998; Boyero, 2003).

On the other hand, already in the 1920s, habitat was clearly seen as a filter for biological traits, and patterns in these traits were related to spatial habitat variability as well as to disturbance (Statzner et al., 2001). These ideas were merged by Southwood (1977) in the "habitat templet concept". Southwood (1977, 1988) postulates that spatio-temporal habitat variations provides a "templet" against which differences in fundamental life history and other species traits result in differential survival and reproduction. Consequently, over evolutionary time, there should be a correspondence between life history traits and habitat characteristics. Townsend & Hildrew (1994) developed their "River Habitat Templet" by predicting trends of traits across spatio-temporal variability gradients. They predicted that organisms in habitats with high temporal and low spatial variability would be more resilient and/or more resistant than organisms in habitats with opposite conditions. Temporal variability refers to the frequency of disturbances whereas spatial variability refers to the abundance of refuges buffering the effect of disturbances (Townsend & Hildrew, 1994; Townsend et al., 1997).

Another proposed axis for the habitat template was the "adversity (or favourableness) axis", ranging from harsh to rich habitats (Southwood, 1977). In this context, in

continuously harsh environments, owing to physical (e.g. hot springs) or chemical (e.g. salinity) factors, adapted species would be relatively free of interspecific competition (Hynes, 1970). Greenslade (1983) termed this kind of selective forces as 'beyond K' selection.

Trends of species traits in these habitat templets were usually obvious and often statistically significant (Resh et al., 1994; Scarsbrook & Townsend, 1993; Statzner et al., 1997; Townsend et al., 1997; Usseglio-Polatera, 1994). In a complementary way, the traits of a faunal assemblage can demonstrate some of the characteristics of the environment, presenting clear information on the rate of spatial or temporal changes in their habitats (Usseglio-Polatera et al., 2000)

Poff (1997) proposed a conceptual framework in which environmental factors (acting at hierarchically organized spatial scales) act as "filters" in a top-down way (from large scale geo-climatic constraints to microhabitat features), successively precluding those taxa whose characteristics are not adapted to couple with prevalent environmental characteristics. In this manner, certain combinations of biological traits and life history tactics are assumed to be selected depending on the environmental characteristics of habitats. Thus, to understand the distribution of aquatic organisms and the biological and ecological mechanisms explaining this distribution, we must reveal the links between traits of organisms and environmental limiting factors or "filters" acting at different scales.

Hydrological variation has come to be viewed as an important element of the habitat templet. Accordingly, geographical patterns of hydrological variation among streams can suggest regional-scale differences in ecosystem structure and function (Poff, 1996). The Mediterranean semi-arid regions are characterised by unevenly distributed water resources,

hydrological variability (low rainfall irregularly distributed in time and space), heterogeneous topography and anthropogenic pressure. On the other hand, organisms living in areas that frequently experience major but unpredictable abiotic perturbations (floods, droughts) may respond over evolutionary time by developing morphological, physiological, and/or life-history traits that minimize the impact of, or exploit, the disturbance.

Southeast Spain has a semiarid climate with highly variable rainfall from year to year. Flash floods are major problems in the dry southeast region of Spain and could become worse with climate and land-use change (Hooke and Mant, 2002). The landscape in the Segura River basin ranges from Mediterranean conifer forests in mountain ranges along major nature reserves in the NW, to arid and semi-arid mediterranean shrublands further south-east. The longitudinal gradient in altitude and climate is coupled with a human pressure counterpart. The river network has low populated forested headwaters, intermediately populated agricultural midlands with intense flow regulation, and densely populated cities in the lowlands. The hydrological regime of the rivers, especially of the smaller tributaries in the southeast is intermittent, with the rivers being reduced to permanent pools or drying up completely (Vidal-Abarca et al., 1992, 2004; Mellado et al., 2002). This regime is mainly dependent on climatic conditions, but an increasing demand for water resources due to changes in land-use practices have also modified flow regimes (Vidal-Abarca et al., 2004). Moreover, streams in arid regions often develop complex salinity gradients, a well documented feature in the Segura River basin (Moreno et al., 1997, 2001; Mellado et al., 2002). Some of the tributaries in the south-eastern part of the basin flow across salt-rich rocks such as sedimentary Miocene marls rich in sodium chloride and gypsum and therefore their salinity is naturally high (Moreno et al., 1997; 2001). Saline streams have

marked differences in biotic composition compared to their freshwater counterparts in the same region (Aboal, 1989; Moreno et al., 1997, 2001).

A functional approach relating species traits (life-history and physiological/behavioural traits as well as ecological preferences) to faunal changes and habitat characteristics was used to discriminate among organizational structure of communities in streams of contrasting environments.

We used data from 16 different stream sites classified in four distinctive typologies according to Chapter 1. Thus, our study design incorporated mountain forested streams, small tributaries in agricultural catchments, springs and saline streams. Each stream site was sampled in seven occasions during two consecutive years, so we had a consistent data set to address these questions.

We used a recently described multivariate method, RLQ analysis (Dolèdec et al., 1996), which provides a general solution to the problem of relating species traits to habitat variables (the "fourth-corner" problem described in Legendre et al., 1997). RLQ analysis aims to investigate the relationships between two tables ("R" and "Q") that are constructed according to different statistical units (environmental characteristics and species traits in our case) by way of a third table ("L") that represents the link between them (a species abundance matrix). RLQ is centred on interpretation of the scores of the environmental characteristics of the sites and the species traits in common ordination axes (Ribera et al., 2001). RLQ have not been widely used although it has demonstrated very good results in different studies dealing with bird assemblages in an urban-rural gradient (Dolèdec et al., 1996), grassland functional groups (Barbaro et al., 2000), terrestrial carabid traits and land

disturbance (Ribera et al. 2001) or the searching of indicator traits in forest birds to monitor land use impact (Hausner et al., 2003).

Specifically, we addressed the following questions:

1. Are there differences in the suites of biological and ecological traits exhibited by the macroinvertebrate communities from streams of contrasting environments?

2. Which are the main environmental factors driving those differences and at which spatial scales are acting?

3. Which are the main traits involved in these differences?

4. Is there a match between certain ecological strategies and some corresponding life-history tactics?

5. Are those differences in concordance with the River Habitat Templet and other relevant ecological theories?

Up to our knowledge, this is the first study using the RLQ analysis in stream ecosystems and also the first one dealing with multiple functional descriptions of stream communities across a whole catchment in the Iberian Peninsula. Identifying habitat-traits relationships can improve our understanding of how the environment influences species assemblages at multiple scales, with special relevance for monitoring and assessment of water resources

2. Methods

2.1. Study area and sampling design

The study sites are located in the Segura River catchment, SE Spain (Figure 1). General descriptions of the basin area (geology, climate, etc.) can be found elsewhere (Vidal-Abarca et al., 1992; Mellado et al., 2002). We conducted our study in 16 streams belonging to four
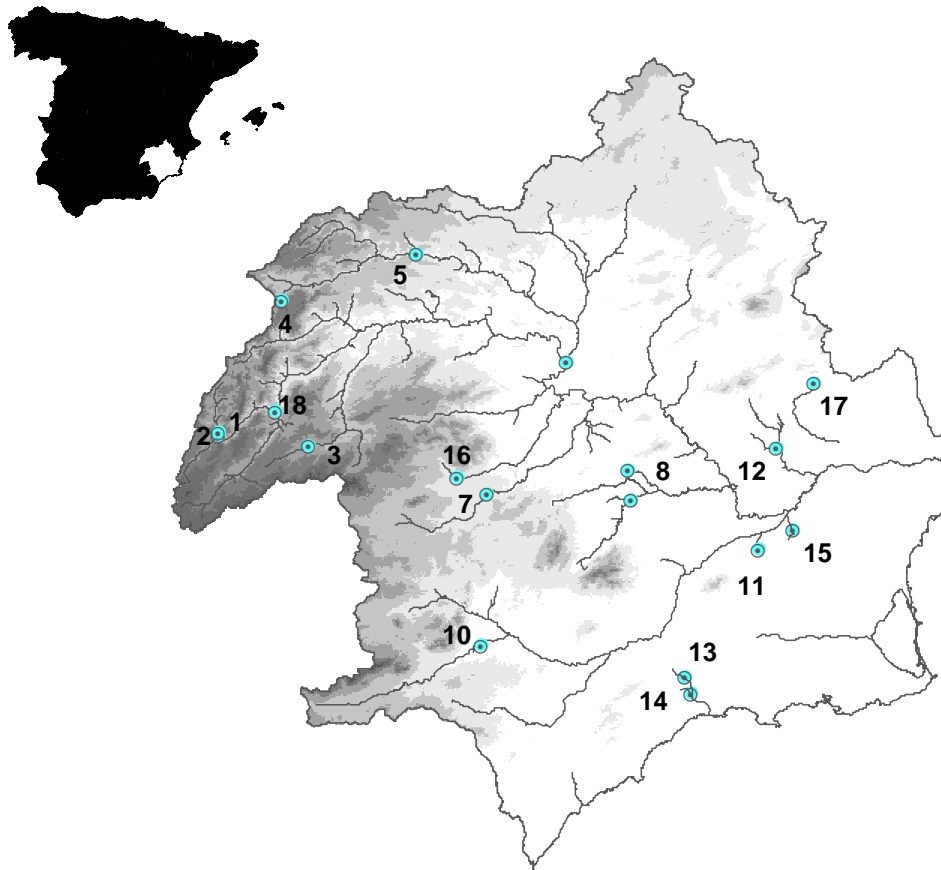
Figure 1. Study area showing the sampling sites and their labels (see text)

different typologies previously defined on the basis of the macroinvertebrate assemblages (Mellado et al., 2002; Chapter 1).

Sites 1, 2, 3 and 4 are first and second-order, high altitude stream reaches (950 to 1040 m) located in mountainous conifer-forested areas in a nature reserve, in the humid NW part of the basin. Sites 5 and 18 are fourth and third-order reaches at medium elevations (650 and 720 m) and like sites 1, 2 and 3, are located in forested areas inside or near the nature reserve limits. All of them were classified as *mountain streams.*

Sites 7, 8, 10 and 16 are medium altitude reaches belonging to four streams (420 to 780 m. and 1st to 3rd order) located in extensive agricultural areas from the middle part of the Segura river basin, thus affected in some degree by agriculture runoffs, water abstractions and diversions for irrigation, livestock grazing and/or recreational activities. These sites were grouped along with a saline lowland stream (site 13) in the *agricultural streams* type.

Sites 11 and 17 are 1st and 3rd order spring-fed stream reaches at low altitudes (350 and 340 m.) with some signs of impairment due to recreational activities or canalization in the first case and to water abstractions and livestock grazing in the other case. These two sites formed the *springs* type.

Finally, sites 12, 13, 14 and 15 are low altitude (100, 60, 140 and 100 m.) saline streams (mean measured conductivities ranged from ca. 9,000 in site 13 up to more than 50,000 $\mu S \cdot cm^{-2}$ in site 12) in semi-arid areas of the south-eastern part of the catchment characterized by salt-rich Miocene marls as the dominant lithology. Sites 13 and 14 are littoral streams, site 12 is a hyper-saline stream that flows into a reservoir, and site 15 is a small intermittent saline tributary of the Segura River near Murcia. Agricultural impacts probably affect these streams to some degree. All these sites were classified as *saline streams*, with the exception of site 13, included in the *agricultural streams* faunal type.

2.2. Macroinvertebrate sampling

105 macroinvertebrate samples were taken from the 16 streams in 7 occasions from 1999 to 2001: April 1999, July 1999, November 1999, February 2000, April to May 2000, July 2000 and December 2000 to February 2001. Some sites were not sampled in all 7 dates due to technical problems or the drying of some sites in summer. One single multi-habitat

semiquantitative kick-sample, as described by Zamora-Muñoz and Alba-Tercedor (1996) was taken in each sampling occasion. In our sampling method, macroinvertebrates are live-sorted in the field from white trays with a portable aspirator trying to collect a representation of the community and getting the maximum diversity as possible, actively searching for rare taxa. The sampling goes on until no new taxa (at family level) are found in the field with successive trays. We preserved samples in 70% ethanol. Invertebrates were identified in the laboratory with the aid of a 6.5-64X Olympus microscope to the lowest taxonomic level (usually genus) except for some dipterans that were identified to families, subfamilies or tribes, Hirudinea (identified to family), Hydracarina, Tricladida, Oligochaeta, Nematoda, Ostracoda, Copepoda and Cladocera. A total of 208 taxonomic units were used in this study (see Appendix 1).

2.3 Biological and ecological traits

We used 62 categories of 11 biological traits and 50 categories of 10 ecological traits to describe the functional composition of invertebrate communities. The traits and their categories used are shown in Table 1 (a, b). The selection of traits and their categories was dictated by the available information. The data-base used here was that in Tachet et al. (2000). It assigned an affinity score (from 1 to 5) of each genus to each trait category using a fuzzy coding approach (Chevenet et al., 1994). For instance, the maximal size achieved by a genus was described as falling into 7 length categories ranging from 2.5 to > 80 mm. If all European records for a genus fell into one length category, it scored the affinity "5" for that category and "0" for all other ones. If most European records fell into one length category but a few lay in a neighbouring category, the genus would score "4" and "1" for the two categories, respectively. Thus, the trait database described the overall European affinity of each genus to each trait category (Statzner et al., 2004). This fuzzy coding

integrates also the possible variations of habits during life stages of a taxon. In the case of missing information for a taxon and a variable, we coded '0' for all modalities. This ensured that in multivariate analyses this 'not documented' taxon was treated with the average profile of all other taxa for the corresponding variable, i.e., its discriminative weight for this particular variable was zero (Chevenet et al., 1994). We recognize the importance of species identifications in stream ecology studies (Lenat and Resh, 2001). However, the functional structure of communities was conserved if higher taxonomic levels were used (Dolédec et al., 1998), especially when analyzed with multivariate methods.

To give the same weight to each taxa and each biological trait in further analyses, affinity scores were standardised to percent affinity so that their sum for a given taxa and a given trait equalled 100 %. For some taxa identified at coarser levels of taxonomic resolution, standardised affinity scores (e.g. Beraeidae for the family level and Oligochaeta for a higher one) were calculated by summing the affinity scores of the genera belonging to this taxonomic group and known from our region and re-scaling the results to a 1-5 scale. The opposite case was also present, for example, when we identified a genus (mostly dipterans) that was not treated at this level in Tachet (2000) but at a coarser one (usually subfamily), we assigned the family or subfamily affinities for that genus. However, there were some southern taxa that could not be used, as they were not treated in Tachet et al. (2000). The snails *Melanopsis* sp., *Mercuria* sp. and *Pseudamnicola* sp. or the beetles *Coelostoma* sp. and *Herophydrus* sp. were some of these taxa. We did not use them in further analyses, neither those individuals which could not be identified to genus due to their small sizes.

Table 1. Biological (a) and ecological (b) traits and categories of taxa considered in the present study (see "Code" for the labelling used in figures for traits categories).

a)

Biological traits	Modalities	Code
Maximal size		
	< 0.25 cm	<0.25
	> 0.25–0.5 cm	0.25–0.5
	> 0.5–1 cm	0.5–1
	> 1–2 cm	1–2
	> 2–4 cm	2–4
	> 4–8 cm	4–8
	> 8 cm	>8
Life cycle duration		
	<= 1 year	1
	> 1 year	>1
Potential No. reproductive cycles per year		
	< 1	<1
	1	1
	> 1	>1
Aquatic stages		
	egg	egg
	larva	lar
	pupa	pu
	adult	ad
Reproduction		
	ovoviviparity	ov
	isolated eggs, free	efr
	isolated eggs, cemented	ec
	clutches, cemented or fixed	cfx
	clutches, free	cfr
	clutches, in vegetation	cv
	clutches, terrestrial	ct
	asexual reproduction	asx
Dissemination		
	aquatic passive	aqp
	aquatic active	aqa
	aerial passive	aep
	aerial active	aea
Resistance form		
	eggs, statoblasts	ee
	cocoons	co
	cells against desiccation	cdes
	diapause or dormancy	dia
	none	no
Food		
	fine sediment + microorganisms	s-m
	detritus < 1mm	fde
	plant detritus > 1mm	cde
	living microphytes	lmph
	living macrophytes	lMph
	dead animal > 1mm	sdan
	living microinvertebrates	lminv
	living macroinvertebrates	lMinv
	vertebrates	ver
Feeding habits		
	absorber	ab
	deposit feeder	depf
	shredder	shr
	scraper	scr
	filter feeder	fil
	piercer (plants or animals)	pier
	predator (carver/engulfer/swallower)	pred
	parasite	par
Respiration		
	tegument	teg
	gill	gi
	plastron	plst
	spiracle (aerial)	spi
Locomotion and substrate relation		
	flier	fli
	surface swimmer	sswim
	full water swimmer	fswim
	crawler	craw
	burrower (epibenthic)	bur
	interstitial (endobenthic)	int

Table 1. (*Continued*)

b)

Ecological traits	Modalities	Code
Temperature		
	Cold (< 15 °C)	Co
	Warm (> 15 °C)	Wa
	Eurythermic	Eur
pH		
	<4	<4
	4-4.5	4-4.5
	4.5-5	4.5-5
	5-5.5	5-5.5
	5.5-6	5.5-6
	>6	>6
Productivity		
	Oligotrophic	Oli
	Mesotrophic	Mes
	Eutrophic	Eu
Saprobity		
	Xenosaprobic	Xen
	Oligosaprobic	Olig
	b-mesosaprobic	ß-mes
	a-mesosaprobic	a-mes
	Polysaprobic	Poly
Salinity		
	Fresh water	Fresh
	Brackish water	Brack
Altitude		
	Lowlands (< 1000 m)	Low
	Piedmont level (1000-2000 m)	Pied
	Alpine level (> 2000 m)	Alp
Longitudinal distribution		
	Crenon	Cre
	Epirhithron	Epir
	Metarhithron	Metar
	Hyporhithron	Hypor
	Epipotamon	Epip
	Metapotamon	Metap
	Hypopotamon	Hypop
	Outside river system	Out
Transversal and vertical distribution		
	River channel	Chan
	Banks, connected side-arms	Bank
	Ponds, pools, disconnected side-arms	Pond
	Marshes, peat bogs	Marsh
	Temporary waters	Temp
	Lakes	Lak
	Ground waters	Subt
Microhabitat		
	Flag, boulder, cobble, pebble	Roc
	Gravel	Gra
	Sand	Sand
	Silt	Silt
	Macrophytes	Maph
	Microphytes	Miph
	Twigs, roots	Twig
	Litter	Litt
	Mud	Mud
Current velocity		
	Zero	Stag
	Slow (< 25 cm/s)	Slow
	Moderate (25-50 cm/s)	Mod
	Fast (> 50 cm/s)	Fast

2.4. Environmental variables

A total of 39 environmental variables were used in this study (Table 2). 12 physicochemical parameters including electric conductivity, discharge, water temperature, suspended solids, dissolved oxygen, pH, alkalinity and nutrient contents were measured in each sampling occasion. 7 geographical or geomorphologic variables were estimated at each site from topographic maps and a geographic information system: geographical coordinates, elevation, stream order, sub-watershed area upstream from a sampling site and percentage of calcareous and marl/alluvial lithology in that sub-watershed. Additionally, 5 land-use variables were estimated at each sampling site using a geographic information system, the CORINE-land cover database modified to some simple indicative layers (CEC 1993) and a hydrological model that accounted for discharge effects on the influence at the receiving stream site. These parameters were percentages of urbanized, industrial, agriculture/pasture, not-modified and forested plus not-modified areas in the sub-watershed multiplied by a specific correction factor derived from the hydrological model for each site, thus obtaining the final land-use ratios.

As in-stream habitat variables we used a set of 8 metrics included in a physical habitat multi-metric index (IHF) inspired in the British River Habitat Survey (National Rivers Authority, 1995) and the US-EPA rapid bioassessment protocols (Barbour et al., 1999) and fully described by Pardo et al. (2002). These metrics were substrate embeddedness, substrate size heterogeneity, riffle frequency, variability of velocity/depth regimes, shading of the channel, heterogeneity elements (roots, wood, debris dams, etc.) and macrophyte cover heterogeneity as well as the final IHF score. We calculated these metrics in three sampling occasions and averaged values were used. Similarly, as riparian forest variables we used the 4 components included in the QBR index described in Munné et al. (2003) as well

Table 2. Environmental variables used in the present study, organized by variable types, and their respective scores on the RLQ$_b$ and RLQ$_e$ first ordination axes (`Code' is the label used in the figures for environmental variables)

Environmental variable	Variable type	Code	RLQb score	RLQe score
Total suspended solids	*Physicochemical*	SS	-0.059	0.086
Ammonium	*Physicochemical*	Ammo	-0.040	0.038
Nitrite	*Physicochemical*	Nitri	-0.057	0.065
Nitrate	*Physicochemical*	Nitra	**-0.108**	**0.160**
Phosphate	*Physicochemical*	Phos	-0.045	0.060
Alkalinity	*Physicochemical*	Alk	-0.042	0.064
Dissolved oxygen	*Physicochemical*	Ox	0.027	-0.033
Oxygen saturation	*Physicochemical*	Ox%	-0.015	0.029
pH	*Physicochemical*	pH	0.062	-0.085
Water temperature	*Physicochemical*	T	**-0.083**	**0.122**
Electric conductivity (25ºC)	*Physicochemical*	Con	**-0.128**	**0.184**
Discharge	*Physicochemical*	Q	**0.093**	**-0.138**
Longitude (East)	*Geomorphological*	E	**-0.131**	**0.189**
Latitude (North)	*Geomorphological*	N	**0.092**	**-0.143**
% calcareous subcatchment area	*Geomorphological*	calc	**0.115**	**-0.166**
% marls subcatchment area	*Geomorphological*	marl	**-0.098**	**0.139**
Altitude	*Geomorphological*	Alt	**0.113**	**-0.166**
Stream order (Strahler)	*Geomorphological*	Ord	-0.005	0.006
Subcatchment area	*Geomorphological*	Area	0.012	-0.021
Average channel width	*Instream habitat*	Wid	0.067	-0.098
Substrate type	*Instream habitat*	Sub	0.061	-0.088
Fluvial Habitat Index (IHF)	*Instream habitat*	IHF	0.010	-0.007
IHF - Embeddedness	*Instream habitat*	emb	-0.058	0.077
IHF - Riffle frequency	*Instream habitat*	rf	0.043	-0.040
IHF - Substrate heterogeneity	*Instream habitat*	sb	-0.047	0.077
IHF - Velocity-Depth regimes diversity	*Instream habitat*	vd	0.047	-0.056
IHF - Shadded channel	*Instream habitat*	sh	0.056	-0.099
IHF - Heterogeneity elements	*Instream habitat*	het	0.058	-0.088
IHF - Macrophyte cover	*Instream habitat*	co	-0.017	0.033
Riparian forest quality index (QBR)	*Riparian forest*	QBR	**0.083**	**-0.121**
QBR - vegetation cover	*Riparian forest*	QBRc	0.004	0.000
QBR - vegetation cover structure	*Riparian forest*	QBRs	0.063	-0.093
QBR - riparian forest quality composition	*Riparian forest*	QBRq	**0.092**	**-0.139**
QBR - riparian forest naturalness	*Riparian forest*	QBRn	0.051	-0.076
Urban use ratio (subcatchment)	*Land use*	Rurb	-0.066	0.087
Industrial use ratio (subcatchment)	*Land use*	Rind	-0.033	0.048
Agricultural use ratio (subcatchment)	*Land use*	Ragr	**-0.106**	**0.149**
Natural use ratio (subcatchment)	*Land use*	Rnat	**0.116**	**-0.163**
Percentage natural subcatchment area	*Land use*	Nat	**0.112**	**-0.156**

as the final QBR score. This index has been applied recently in the study area and some modifications were proposed for its use in semi-arid catchments (Suárez & Vidal-Abarca, 2000). It is based on four additive metrics: riparian vegetation cover (proportion of the riparian area covered by trees and shrubs), cover structure (proportion of riparian vegetation composed by trees and shrubs separately), riparian quality (number of trees or shrub species and absence of introduced species, and other human impacts in riparian vegetation) and channel alterations (Absence of human impacts altering channel form). It also takes into account differences in the geomorphology of the river from its headwaters to the lower reaches. We calculated the QBR index metrics in a single sampling occasion, as we did not detect any change in riparian forest at our study sites during the study period.

2.5. Statistical analyses

Three separate ordinations of the R (environmental variables), L (taxa composition), and Q (taxa traits) tables were performed prior to the RLQ analysis (Figure 2). First, correspondence analysis (CA) was conducted on the taxa composition table (L), providing an optimal simultaneous ordination of samples and taxa. The samples and taxa scores (or coordinates) will later on function as links between the R and Q tables, as samples are shared by the R and L tables and taxa are shared by the Q and L tables. The second step in this procedure was to investigate relationships between samples and environmental variables (i.e., R table). A principal component analysis (PCA) was conducted, with sample score in L as row weights allowing R to be linked to the L table. To interpret this analysis we used the loadings of each environmental variable on the components of the PCA.
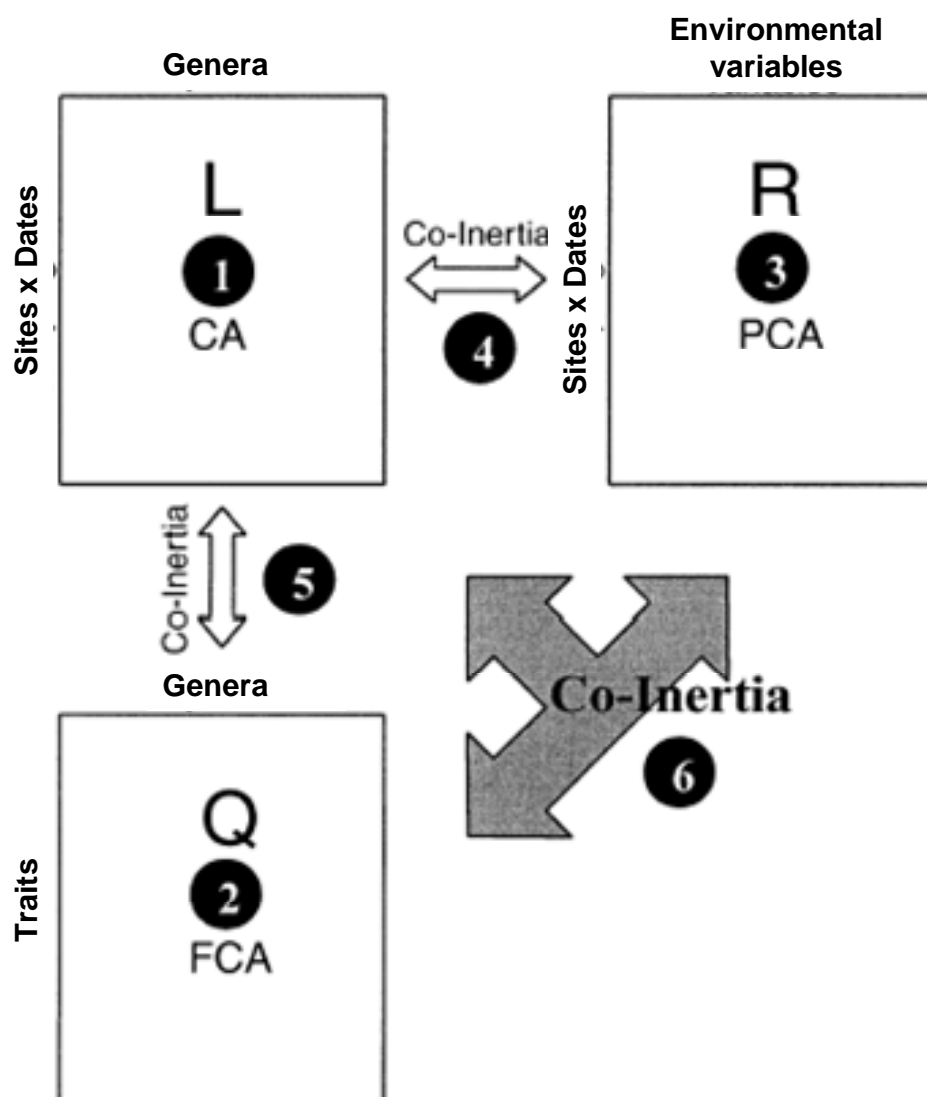
Figure 2. A conceptual diagram showing the RLQ analysis procedure (Modified from Hausner et al., 2003)

Next, the trait tables (Q_b and Q_e) were investigated by fuzzy correspondence analysis (FCA; Chevenet et al., 1994). Each Q table was translated to fuzzy variables, wherein affinity scores are used to calculate frequency distribution of categories within traits. The modalities of these frequencies were then used for joint scaling of taxa and traits, where the taxa scores in the L table were used now as row weights in order to link the Q table with

the L table. To select traits which best explained the variance on a given axis, we arbitrarily used correlation ratios (i.e. percentage of extracted between-category variance, see Chevenet et al., 1994).

RLQ analysis is a doubly constrained ordination that could be compared to the unconstrained ordination provided by CA. This comparison would indicate how much of the macroinvertebrate community structure as represented in CA is associated with environmental variables and biological/ecological traits. RLQ analysis combines the three separate analyses so as to maximize the co-variation between environmental variables and taxa traits. In other words, the sample scores in the R table constrain the sample scores in the L table, whereas the taxa scores in the Q table constrain the taxa scores in the L table. Within these constraints, co-inertia analysis (see Dolédec and Chessel, 1994) selects axes that maximize covariance between the taxa and the sample scores in the L table. In summary, the maximization of covariance results in the best joint combination of the ordination of sites by their environmental characteristics (optimization of the site score variability), the ordination of species by their traits (optimization of the species score variability), and the simultaneous ordination of species and sites (optimization of the correlation between the sites scores and the species scores). As a result, the environmental attributes are related to taxa traits. RLQ takes into account only a fraction of the total variance.

The significance of the relationship between the environmental attributes (R) and taxa traits (Q) was investigated by a permutation test (Dolédec et al., 1996). The results of 1000 random permutations between the R and Q table were compared with the total inertia obtained in the RLQ analysis (the trace). The number of permutations that generated higher values than the observed total inertia set the statistical significance of relationship.

In order to evaluate the strength of these relationships, the constrained ordination resulting from the three tables in the RLQ analysis was compared with the unconstrained separate analyses of R, L, and Q. That is, the percentage of the variation in each separate analysis taken into account by the RLQ analysis was calculated for the major axes in the R, L, and Q tables. Finally, in order to assess the relative contribution of each environmental variable to the new ordination we used the factor loadings on the main RLQ axes. Similarly, the main biological-ecological traits responsible of the observed variability on the RLQ ordination were assessed by their correlation ratios (Chevenet et al., 1994). All analyses were made using the R software, version 1.9.0 (R Development Core Team, 2004).

3. Results

3.1. Separate ordination of the data tables

3.1.1. Faunal table (L)

Total variability (inertia) in the macroinvertebrate data was 4.07, and the first four axes of the Correspondence Analysis (CA) could explain 27.3 % of this variability. The two first axes of the CA of the taxa abundance matrix (log-transformed) accounted for only 18.3 % of the total variance. The first axis explained near 12 % while the second axis explained about 7 % (Table 3). Additional axes were not considered in the RLQ analysis. The first eigenvalue corresponded to a canonical correlation equal to 0.69 (or the square root of 0.47). This value is the best possible correlation within the species abundance matrix. The projection of samples on the factorial map showed a clear arch effect (Figure 3), but also a clear grouping of the samples in the four stream types previously defined. However, there was a small overlap between *springs* and *agricultural streams*.

Table 3. Results of the separate analyses involved in both RLQb and RLQe analyses.

CA-Taxa-Samples table (Table "L")				
Eigenvalues (1 to 4)	0.47	0.27	0.20	0.16
Variance (%)	11.65	6.61	5.02	4.02
PCA-Environmental variables (Table "R")				
Eigenvalues (1 to 4)	11.81	4.43	3.25	3.18
Variance (%)	30.29	11.37	8.34	8.16
FCA-Biological traits table (Table "Qb")				
Eigenvalues (1 to 4)	0.22	0.20	0.18	0.13
Variance (%)	10.84	9.86	8.69	6.41
FCA-Ecological traits table (Table "Qe")				
Eigenvalues (1 to 4)	0.18	0.10	0.05	0.04
Variance (%)	27.88	15.32	7.55	5.61

3.1.2. Environmental table (R)

The two first axes of the PCA of the environmental characteristics of the sites accounted for 41.7% of the total variance, with axis 1 explaining more than 30% and axis 2 about 11% (Table 3). The first axis was mainly positively associated (loadings higher than 0.75) with the agricultural land use ratio, nitrates, percentage of marl/alluvial sub-basin, the eastern coordinate and electric conductivity, and negatively associated with the naturalized land use ratio, the percentage of calcareous sub-basin, the forested-naturalized land use ratio, elevation, the riparian forest QBR index and the riparian quality sub-index (Figure 4). These were all variables highly correlated with elevation ($r > 0.59$ in all cases). Elevation was also highly correlated with longitude, latitude, predominant geology and land use, something to be taken into account when interpreting the results (see discussion).
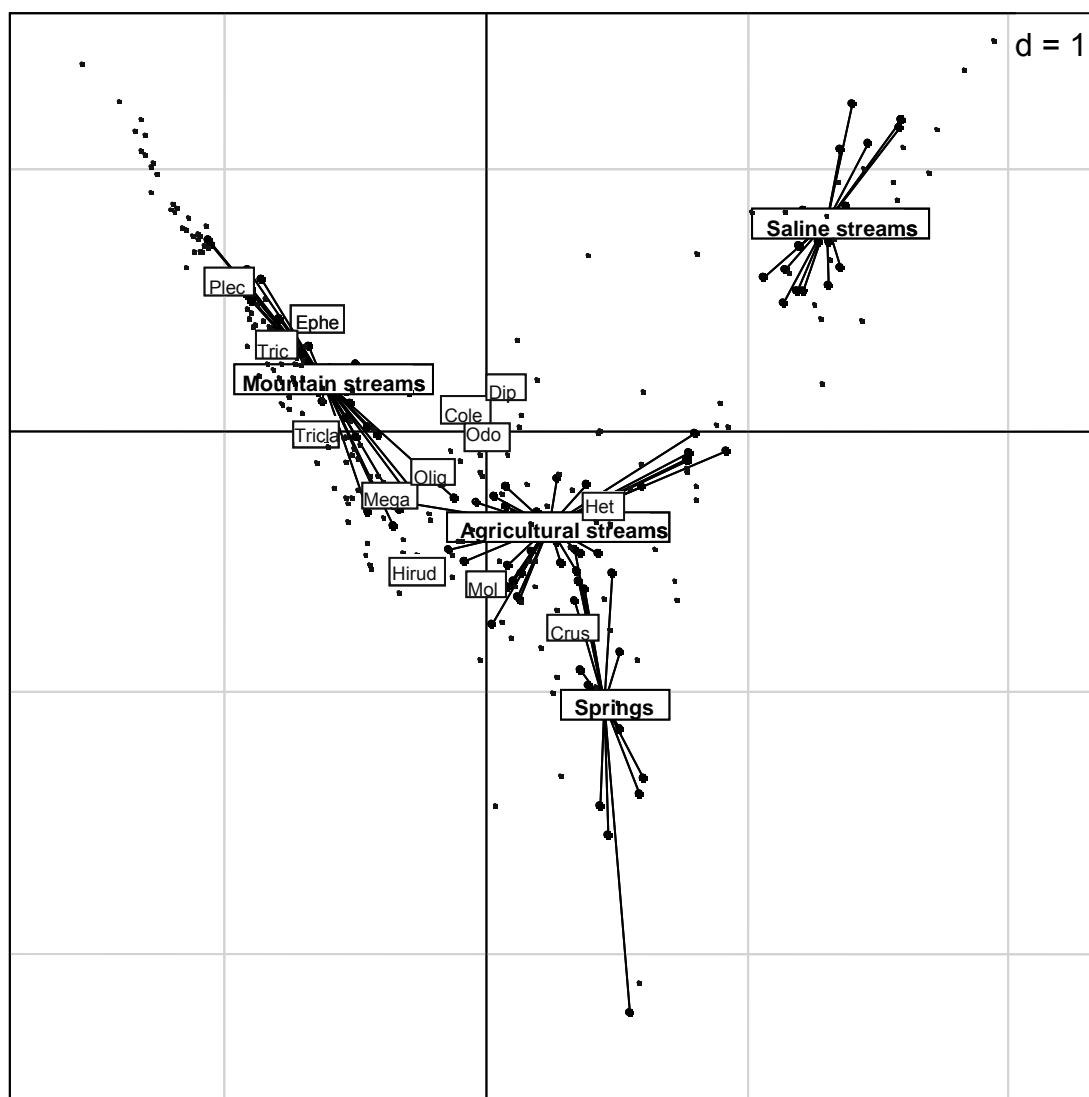
Figure 3. Correspondence analysis biplot showing the samples grouped by stream types and taxa grouped by main orders. Labels are in the average positions of taxa and sites.
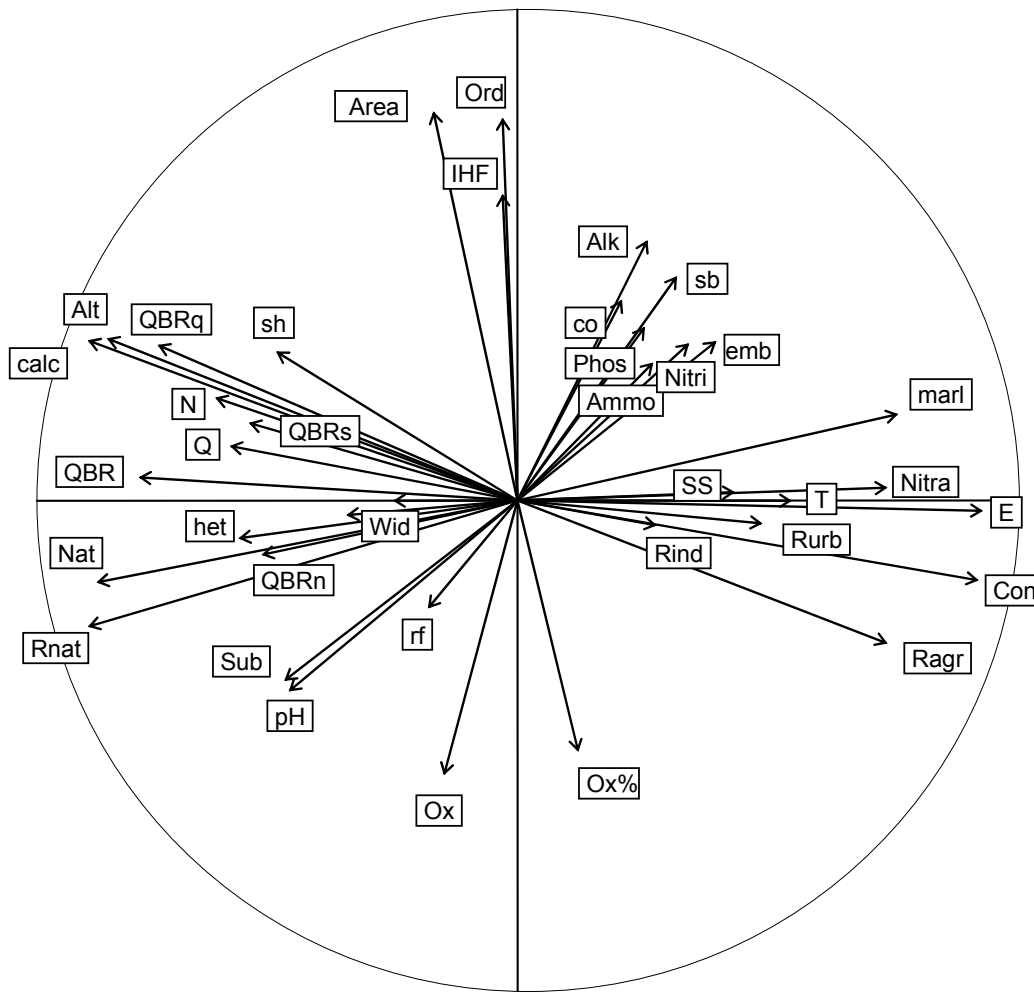
Figure 4. Principal component analysis (PCA) correlation circle of the 39 parameters. Short vectors have not been labelled for clarity. Environmental variable codes are shown in Table 2.

The second axis had the highest positive correlations (loadings >0.63) with sub-watershed area, stream order and with the in-stream habitat quality index (IHF) and negative relations (loadings > 0.5) with oxygen concentration and saturation. The ordination of samples on the two first components space is showed in Figure 5. *Mountain streams* and *saline streams* were clearly isolated along the first axis, with the first group occupying negative positions and the later presenting the highest values. Meanwhile *agricultural streams* and *springs* samples occupied central positions and showed a moderate overlap between them, with site 17

samples grouped with the *agricultural streams* samples. The second component (only 11 % of absorbed variance) of the PCA does not discriminate stream types, but it separated sites
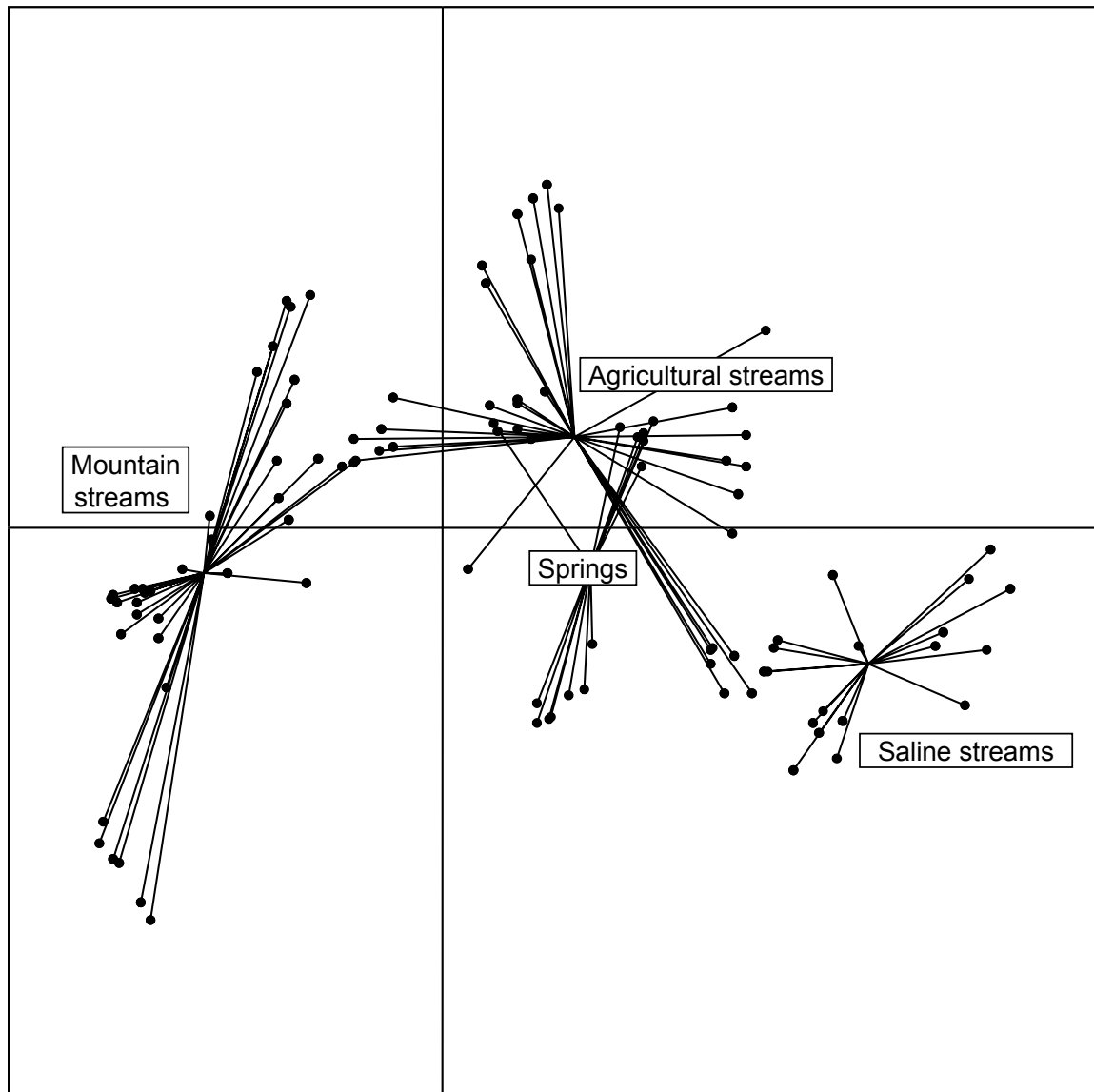
Figure 5. PCA plot of the samples, grouped by stream types. Lines link samples to their stream types. Labels are plotted on the average positions occupied by the sites from each group.

within stream types (all but saline streams) mainly on the base of stream size (catchment area, order). Site 4, a karstic spring-fed stream in the *mountain streams* group occupied the

most negative values, followed by site 11 and site 13, from *springs* and *agricultural streams* respectively, and *saline streams*.

3.1.3. Biological traits table (Q_b)

The two first axes of the fuzzy correspondence analysis (FCA) on the biological traits-taxa matrix accounted for 20.71 % of the total variance (total inertia 2.05, Table 3). The first axis was mainly correlated with feeding habits and type of food and to a lesser degree with maximal size, life cycle duration and reproductive strategy, although for the first and latter the highest correlation was with axis two (Figure 6). The second axis was principally correlated with the type of respiration, the reproductive strategy and the maximal size. Modalities of the variables feeding habits were also separated on this axis but not as much as on the first axis (Figure 6). The third axis (8.7 % of variance) was positively correlated with reproduction and feeding habits, so that it did not add new information to the analysis so that it was not considered further.

The plot of taxa (grouped as orders) on the two first FCA axes is shown in Figure 7. Long-lived taxa with higher maximal sizes occupied positive values along both axes. They were predators feeding on living macroinvertebrates, laying isolated eggs (both free and cemented) and with gill respiration. Invertebrates in this group comprised Odonata, the Megaloptera *Sialis* sp., some taxa in Plecoptera (*Perla* sp. and *Dinocras* sp.), Trichoptera (Rhyacophilidae), Ephemeroptera (*Ephemera* sp.) or crustaceans (crayfish) as well as Tricladida and Hirudinea. On the contrary, the upper-left quadrant (negative values on F1 and positive on F2) was mainly occupied by medium sized filterers and deposit-feeders feeding on detritus, fines and microorganisms. They were short-lived taxa (1 year or less), and the typical reproduction was sexual with free clutches. The respiration mechanisms were tegument or gills. Most of the Ephemeroptera, Trichoptera, Diptera and Plecoptera
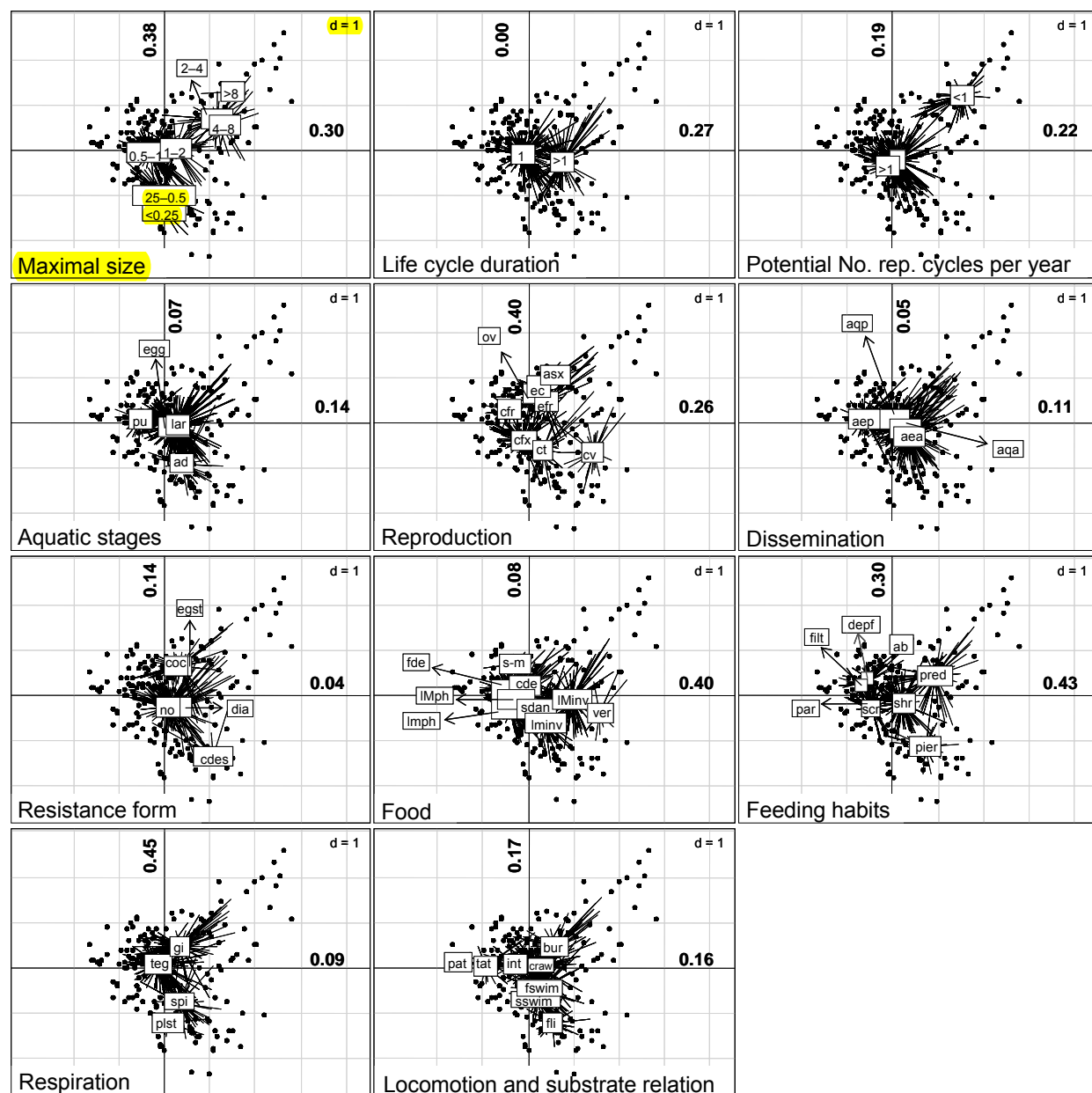
Figure 6. Distribution of modalities (white labels) of the biological traits on the F1-F2 factorial plane of the fuzzy correspondence analysis (FCA). Each modality label was positioned at the weighted average of the taxon positions (solid squares, compare Fig. 7) representing this modality. Numbers indicated the correlation ratios with axis F1 (horizontal numbers) and F2 (vertical numbers). Some labels have been moved for clarity and their actual position is indicated by arrows. Ordination plot scale (i.e. the longitude of the main divisions, "d") is indicated. Codes used for trait modalities are shown in Table 1a.
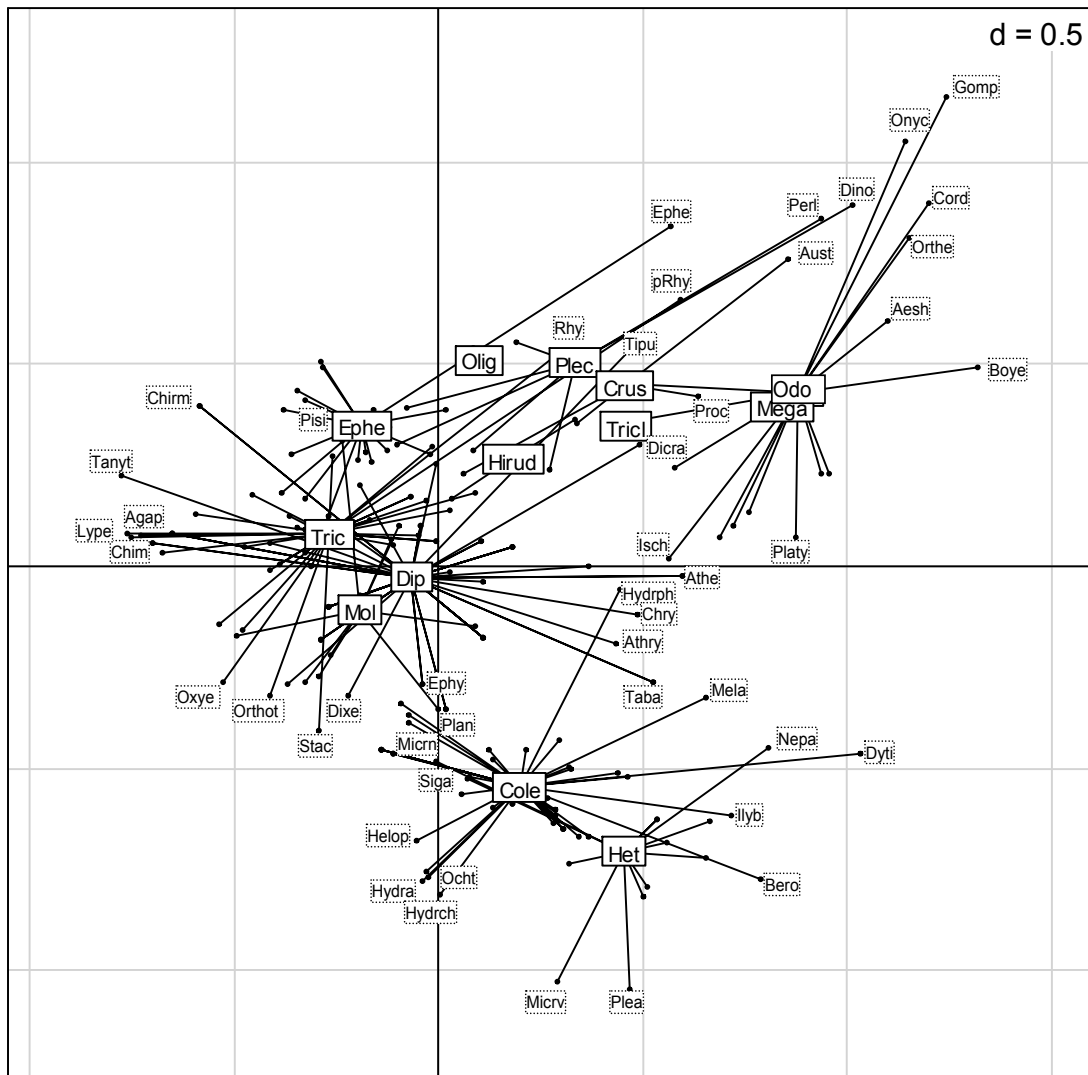
Figure 7. Distribution of macroinvertebrate taxonomic units on the F1-F2 factorial plane of the fuzzy correspondence analysis on biological traits. Taxonomic units (white square labels) were positioned at the weighted average of their taxa (solid circles); lines link taxa to their systematic units. Those taxa having extreme scores relative to their groups are indicated (dotted line square labels). For taxa codes, see Appendix 1. Ordination plot scale (i.e. the longitude of the main divisions, "d") is indicated.

formed the bulge of this group, with some bivalves as *Pisidium* sp. The negative F2 values were typical of smaller taxa with short and long live spans. The normal reproductive strategies were sexual with fixed terrestrial and endophytic clutches. Scrapers feeding on living macrophytes and piercers of small invertebrates or vertebrates were the main functional feeding guilds. Respiration was mainly by plastron or aerial by spiracles.

Coleoptera and Heteroptera mainly composed this group, along with some taxa in Mollusca and Diptera.

3.1.4. Ecological traits table (Q_e)

The two first axes of the fuzzy correspondence analysis (FCA) on the ecological traits-taxa matrix accounted for 43.2 % of the total variance (total inertia 0.65, Table 3). The first axis (27.9 %) was mainly correlated with current velocity preference, transversal and longitudinal distribution and to a lesser degree with productivity preference, saprobity or microhabitat preferendum (Figure 8). The second axis (15.3 %) was principally correlated with temperature preference and more weakly with longitudinal distribution but not as much as on the first axis (Figure 8). The third axis (7.5 % of variance) was also positively correlated with temperature, not adding new information to the analysis so that it was not considered further.

The plots of taxa (classified by taxonomic groups) on the two first FCA axes are shown in Figure 9. Taxa preferring moderate to fast current velocities, mainly inhabiting the main channel or the banks of rithral and crenal sections of streams, had the highest scores on the first ordination axis. They were xenosaprobic and oligosaprobic taxa showing preference for oligotrophic or mesotrophic waters. Microhabitat preferences were mainly rocky substrates, gravel as well as twigs and roots or sand. The plecopterans, a number of mayflies (e.g. *Epeorus* sp., *Rhitrogena* sp.) and caddisflies (e.g. *Pararhyacophila* sp., *Chimarra* sp.) along with some Elmidae, the European crayfish *Austropotamobius* sp. or some dipterans such as *Dixa* sp. or the Empididae showed the highest scores (Figure 9).
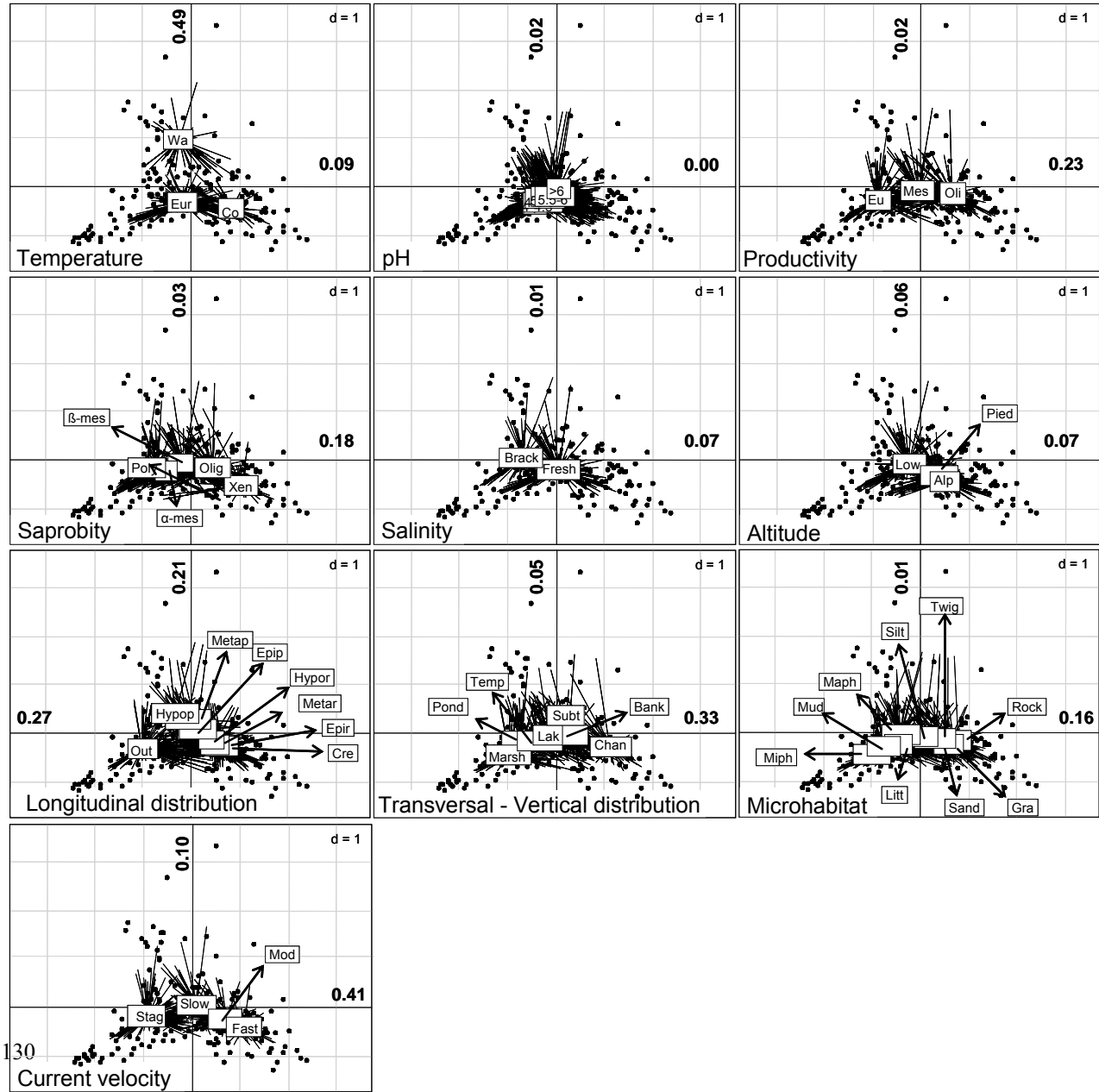
Figure 8. Distribution of modalities (white labels) of the ecological traits on the F1-F2 factorial plane of the fuzzy correspondence analysis (FCA). Each modality label was positioned at the weighted average of the taxon positions (solid squares, compare Fig. 9) representing this modality. Numbers indicated the correlation ratios with axis F1 (horizontal numbers) and F2 (vertical numbers). Some text labels have been moved for clarity and their actual position is indicated by arrows. Ordination plot scale (i.e. the longitude of the main divisions, "d") is indicated. Codes used for trait modalities are shown in Table 1b.

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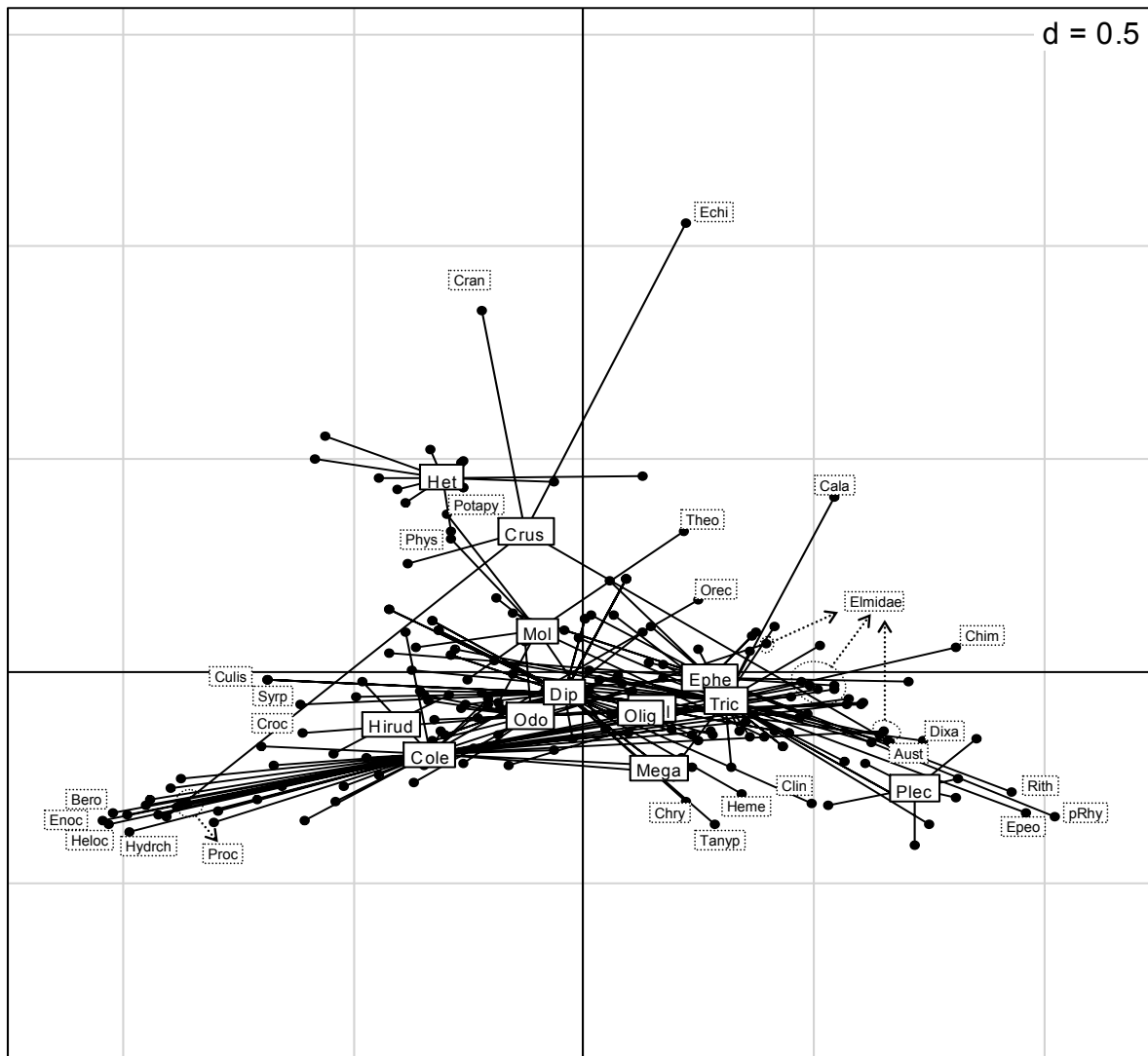
Figure 9. Distribution of macroinvertebrate taxonomic units on the F1-F2 factorial plane of the fuzzy correspondence analysis on ecological traits. Taxonomic units (white square labels) were positioned at the weighted average of their taxa (solid circles); Those taxa having extreme scores relative to their groups are indicated (dotted line square labels). For taxa codes, see Appendix 1. Ordination plot scale (i.e. the longitude of the main divisions, "d") is indicated.

In contrast, negative values along this axis were occupied by stagnant to slow waters taxa. They live in ponds, pools, marshes or temporary waters, showing clear preferences to habitats outside the river system or the potamal zone. The nutrient status preferendum was from eutrophic to mesotrophic and they were quite tolerant to organic pollution (polysaprobity and mesosaprobity).

Preferred microhabitats counted microphytes, mud, litter and macrophytes. A number of water beetles were located in this zone, mainly hydrophilids (as *Enochrus* sp., *Berosus* sp. or *Helochares* sp.) and some Dytiscidae. Some dipterans as *Culiseta* sp. or the Syrphidae, the introduced crayfish *Procambarus* sp. and the Anisoptera *Crocothemis* sp. presented low scores too (Figure 9).

Warm waters taxa preferring lower stream reaches (the hypopotamon and metapotamon) occupied the highest positive values along the second ordination axis and moderately negative values along axis 1. They were the Heteroptera along with two crustaceans (*Echinogammarus* sp. and *Crangonyx* sp.) and two gastropods (*Physella* sp. and *Potamopyrgus* sp.) (Figure 9).

3.2. RLQb: Joint analysis of biological traits, environmental variables, and taxonomic composition

The first two axes of the RLQ_b analysis extracted 78.3 % and 8.9 % respectively, of the total variance of the matrix that crosses the site environmental characteristics and the biological traits of the genera (Table 4). The 1000 random permutations test resulted highly significant (estimated P = 0.001) which is interpreted as a strong evidence of the link between biological traits and environmental attributes. The first RLQ_b axis accounted for 98.5 % of the potential variability for the first axis in the separated PCA of the environmental variables (i.e., the ratio between the variance of the habitat characteristics accounted for in RLQ_b (11.6) and that of the separate analysis (11.8) is 0.985, see Table 3 and Table 4). Similarly, it took into account 51.9 % of the potential variability for the first axis in the separate analysis of the biological traits. The two new sets of sites and taxa scores had a correlation of 0.40 along the first RLQ_b axis (Table 4), a value to be compared

Table 4. Summary of the biological traits RLQ (RLQb) and the ecological traits RLQ (RLQe) analyses.

RLQ$_b$ analysis (biological traits)		
Eigenvalues (1	0.21	0.02
Variance (%)	78.30	8.90
Covariance	0.46	0.15
R/RLQ	eig 1	eig 1+2
Inertia	11.64	14.80
Ratio (%)	99	91
L/RLQ	eig 1	eig 2
Correlation	0.40	0.24
Ratio (%)	58	47
Q/RLQ	eig 1	eig 1+2
Inertia	0.12	0.24
Ratio (%)	52	58
RLQ$_e$ analysis (ecological traits)		
Eigenvalues (1	0.44	0.00
Variance (%)	96.13	1.93
Covariance	0.66	0.08
R/RLQ	eig 1	eig 1+2
Inertia	11.65	14.76
Ratio (%)	99	91
L/RLQ	eig 1	eig 2
Correlation	0.47	0.20
Ratio (%)	68	39
Q/RLQ	eig 1	eig 1+2
Inertia	0.17	0.24
Ratio (%)	94	85

to the highest possible correlation between sites and taxa, given by the square root of the first eigenvalue of the CA (0.69, see Results: Separate ordination). Thus, the first RLQ$_b$ axis explained about 58 % of the variability of the faunal table (L). The covariance between the new sets of scores for the sites (computed from their environmental characteristics) and the species (computed from their biological traits), which is optimized by the first RLQ$_b$ axis, was equal to 0.46 (Table 4, obtained from square root of the first eigenvalue 0.21). In contrast, this value was very low on the second axis (0.15). The ordination axes obtained with RLQ$_b$ and those obtained with the individual analyses were compared in Figure 10a). The new RLQ axes were, as expected, much related to the environmental PCA axes. By

contrast, the RLQ_b axes did not stand for the information given by the three first axes of the separate analysis of the biological traits, but a combination of them (Figure 10a). Because of the low variance explained by the second RLQ_b axis, this was not further considered.

Correlation ratios obtained for the biological traits and the first RLQ_b axis are presented in Table 5. Maximal size, reproductive strategy, respiration and resistance form, were the main attributes explaining the observed biological variability linked to the environmental trends.

Environmental variables loadings on the first RLQb axis are presented in Table 2. Nitrate, water temperature, electric conductivity, eastern longitude and agricultural use were negatively correlated with the first RLQb axis, while calcareous geology, altitude, riparian quality, natural land use and northern latitude were the main positive correlates.

Taxa, biological trait modalities and sampling sites, as well as environmental variables scores on the first RLQb axis were plotted to summarize the results derived from the analysis (Figure 11). To facilitate the interpretation of the graph, taxa scores were plotted according to higher taxonomic groups (usually order) and environmental factors were grouped in physicochemical, in-stream habitat, riparian forest, geo-morphological and land use variables (Table 2). Samples were also classified by stream types. Looking at the positive extreme of the RLQ axis gradient (Figure 11), it is apparent that sites within forested and/or naturalized catchments tend to have well preserved riparian forests (QBR, QBRq), high riffle frequency, rocky substrates, shaded channels and heterogeneity elements

a)

R axes projections on RLQ-biotraits axes

Q axes projections on RLQ-biotraits axes

b)

R axes projections on RLQ-ecotraits axes

Q axes projections on RLQ-ecotraits axes
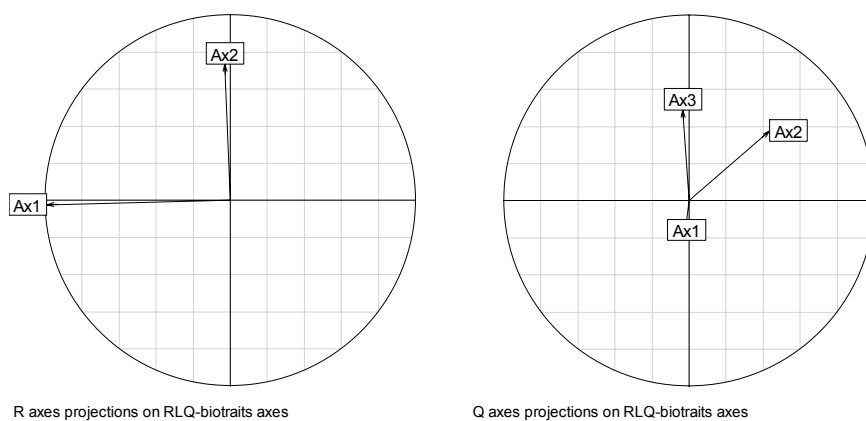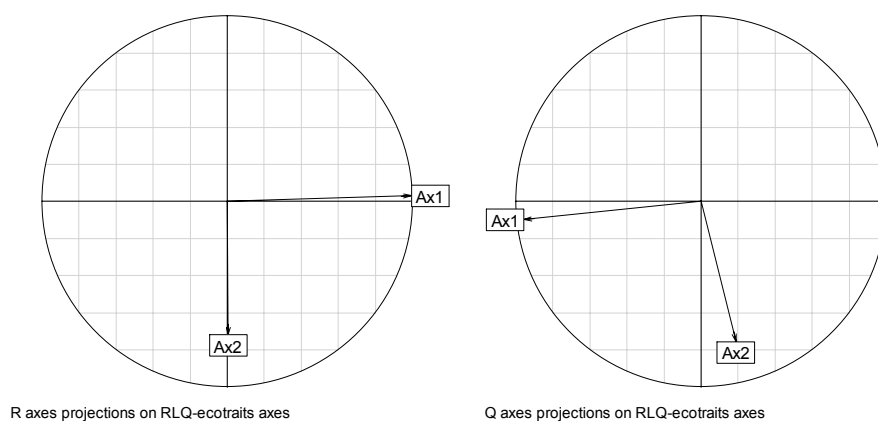
Figure 10. Projection of the axes of each separate analysis on the first two RLQ ordination axes. The graphs on a) correspond to the joint analysis of the environmental data (left, R axes) and the biological traits (right, Q axes). The graphs on b) correspond to the joint analysis of the environmental data (left) and the ecological traits (right). Numbers refer to the number of axes selected in each of the separate analysis (see Results).

Table 5. Biological and ecological traits correlation ratios with their respective first RLQ axes.

Ecological trait	Correlation Ratio
Temperature	**0.54**
pH	0.13
Productivity	**0.47**
Saprobity	0.36
Salinity	0.21
Altitude	0.31
Longitudinal distribution	**0.44**
Transversal and vertical distribution	**0.48**
Microhabitat	0.32
Current velocity	**0.55**

Biological trait	Correlation Ratio
Maximal size	**0.81**
Life cycle duration	**0.61**
Potential no. reproductive cycles per year	**0.61**
Aquatic stages	0.20
Reproduction	**0.76**
Dissemination	0.25
Resistance form	**0.67**
Food	0.28
Feeding habits	0.54
Respiration	**0.74**
Locomotion and substrate relation	0.46

as twigs, roots or debris dams, and a variety of velocity/depth regimes. They are located at high elevations in limestone geology catchments of the humid north western part of the basin, being hard-water sites with relatively high discharge and well oxygenated waters. Samples on this position matched the *mountain streams* type sites (Figure 11). Distinctive biological traits (Figure 11) characterizing the faunal assemblages are: big sizes (2 to 4 cm and 4 to 8 cm classes) and semivoltinism; sexual reproduction laying cemented eggs as reproductive strategy; eggs and statoblasts as resistant stages; relative tendency to passive and active aquatic dispersion; plant debris or fine sediment and microorganisms consumers, with scrapers and filters as the main functional feeding guilds; tegument respiration; and finally permanent attachment, burrowing or crawling as the principal
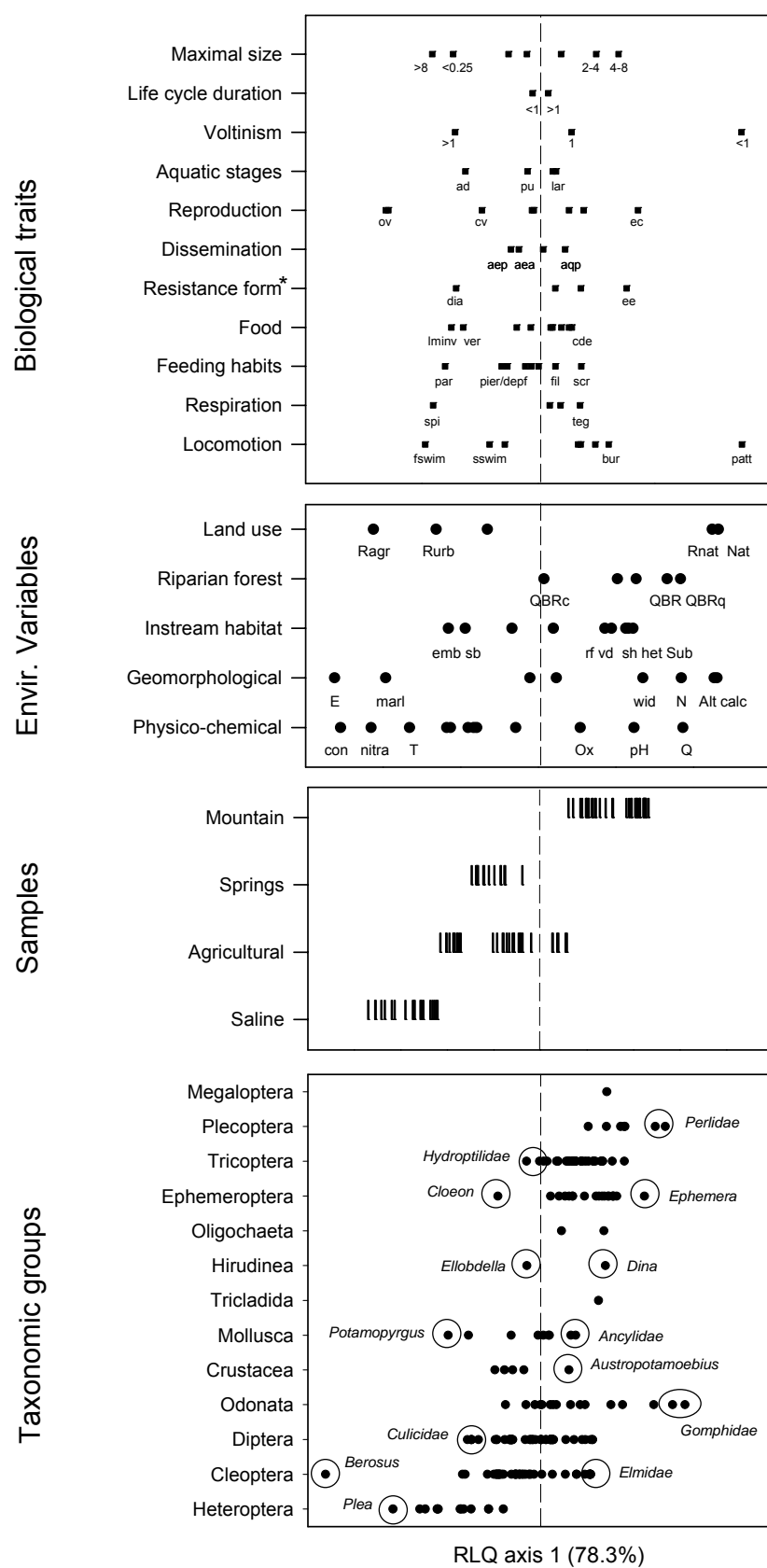
Figure 11. Trait categories, environmental variables, sites and taxa scores along the first axis of the biological traits RLQ analysis. Categories are grouped by trait, environmental factors are grouped by variable type, sites are grouped by stream type and taxa are grouped by faunal groups. Codes can be found in Tables 1(traits) and Table 2 (environmental variables)

locomotion and substrate relationships (Figure 11). All the Plecoptera and most of Ephemeroptera and Trichoptera (EPT taxa) occupied this right side of the axis (Figure 11), along with *Ancylus* sp., *Ferrisia* sp., *Austropotamobius* sp., anisopterans as Gomphidae and *Cordulegaster* sp., and most Elmidae. Contrastingly, sites located in the semi-arid south-eastern part of the study area are characterized mainly by agricultural and land-uses and also included the most urbanized catchments. Dominant geologies are marls with quaternary alluvial deposits and elevation is low at most of sampling sites. Riparian forests and stream channel morphology, as evaluated with the QBR index and its sub-metrics, are not of good quality (presence of introduced species and human alterations in vegetation and/or channel form). Among in-stream habitat variables, high substrate embeddedness, visually estimated as the degree of interstitial filling of the substratum by fine particles and substrate size heterogeneity were the principal differential features. Physico-chemical attributes included high conductivities and nitrate content, high temperature and high suspended solids content. Samples from sites classified as s*aline streams* matched this environmental features in the graph, being located at the negative extreme of the gradient. Common biological traits exhibited by the macroinvertebrate communities inhabiting these systems were: the highest as well as the smallest sizes (<0.25 and >8 cm), polyvoltinism, aquatic adults, oviparity and endophytic clutches; relative affinity for aerial dispersion, cells against desiccation and diapause or dormancy as resistance strategies, living microinvertebrates or vertebrates as the main food sources, parasitic or predatory (piercers) feeding behaviour, aerial respiration through spiracles and swimming locomotion, (both water column and surface swimmers). The majority of the Heteroptera, the hydrophilid *Berosus* sp. (and to a lesser degree *Haliplus* sp. or *Ilybius* sp.) some dipterans in Culicidae and snails as *Potamopyrgus* sp. dominated the negative side of the RLQ_b axis. Among the Ephemeroptera, the genus with the lowest score was *Cloeon* sp.

3.3. RLQe: Joint analysis of ecological traits, environmental variables, and taxonomic composition

The first two axes of the RLQ_e analysis extracted 96.1 % and 1.9 % respectively, of the total variance of the matrix that crosses the site environmental characteristics and the ecological traits of the genera (Table 4). Again, the 1000 random permutations resulted in a highly significant link between environmental variables and ecological traits through the species abundance (estimated $P = 0.001$). Similarly as what occurred analyzing biological traits, the first axis of the RLQ_e analysis accounted for 98.6 % of the potential variability for the first axis in the separated PCA of the environmental variables of the habitats (i.e., the ratio between the variance of the habitat characteristics accounted for in RLQ_e (11.7) and that of the separate analysis (11.8) is 0.986, see Tables 3 and 4). However, the proportion of the potential variability for the first axis in the separate analysis of the ecological traits (94 %) was much higher than the one obtained using biological traits (52 %). The two new sets of sites and taxa scores had a correlation of 0.47 along the first RLQ_e axis (Table 4), which again has to be compared to the square root of the first eigenvalue of the CA of the taxonomic abundance table (0.69, see Results: Separate ordinations). Thus, the first RLQ_e axis explained about 68 % of the variability of the faunal table (L) obtained by CA. The covariance between the new sets of scores for the sites (computed from their environmental characteristics) and the genera (computed from their ecological traits), which is optimized by the first RLQ axis, was equal to 0.66 (Table 4, obtained from square root of the first eigenvalue 0.44). In contrast, this value was again very low on the second axis (0.09). The ordination axes obtained with RLQ_e and those obtained with the separate analysis of the individual tables were compared in Figure 10b). The structure of the environmental matrix described by the RLQ_e axes was, as expected, very close to that of the separate PCA. In this occasion, the RLQ_e axes also represented (almost directly) the information given by the two first axes of the separate analysis of the ecological traits. This

result was somehow expected, as our ecological traits (optima for various environmental variables) are directly related to the measured habitat factors, while more subtle relationships were assumed for life-history or behaviour traits. As was the case for the biological traits analysis, the second RLQ_e axis was not considered further due to the very low variance explained (1.9 %).

Correlation ratios obtained for the ecological traits and the first RLQ_e axis are presented in Table 5. Temperature, productivity, longitudinal and transversal distribution as well as current velocity preferendum were the main traits explaining the observed ecological variability linked to the environmental trends.

A similar uni-dimensional plot was constructed where taxa, ecological trait modalities and samples scores, as well as environmental variables loadings on the first RLQ_e axis were plotted to summarize the results (Figure 12). The loadings of environmental variables as well as sites and taxa scores were very similar to those obtained using biological traits, although with opposite sign (Table 2) The negative correlation between the 209 genera scores on the two RLQ axes (RLQ_b-1 and RLQ_e-1) was equal to -0.67, while between the 104 site scores and the 39 environmental variables loadings was equal to -0.99 in both cases. For clarity, we change the sign of the axis in this plot, to resemble that obtained for the biological traits analysis. Thus, the same environmental interpretation of the RLQb axis is valid in this case. According to the ecological preferences of the macroinvertebrate fauna, the positive extreme of the RLQ_e axis was characterized by cold-water taxa, preferring oligotrophic systems and with very low tolerance values to organic pollution (xenosaprobic and oligosaprobic taxa). They tended to prefer high altitude reaches in the epirithral and metarithral sections, where they usually occupy the main channel. Preferred habitats were rock, gravel or sand substrates with moderate to fast current velocity.
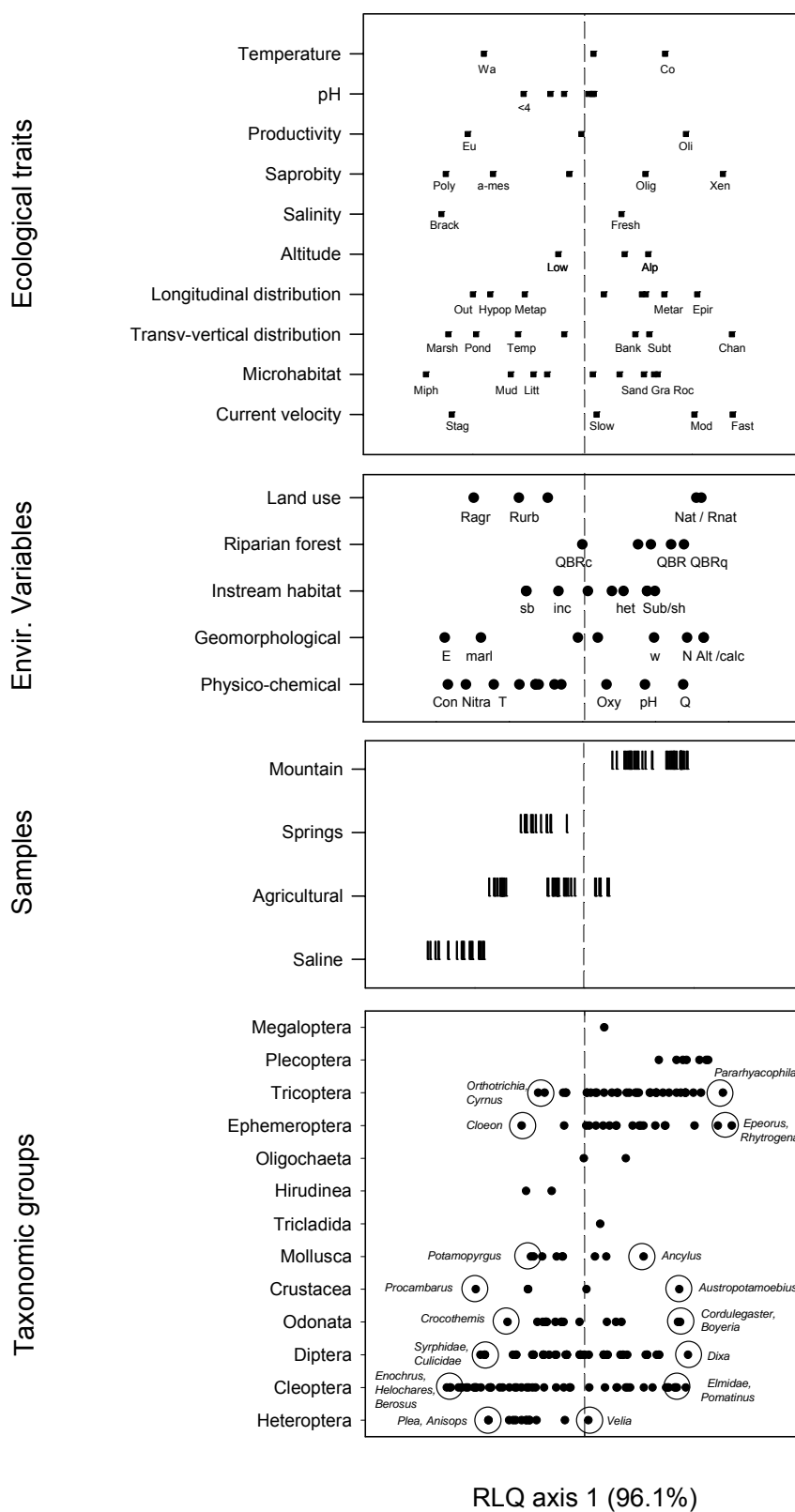
Figure 12. Trait categories, environmental variables, sites and taxa scores along the first axis of the ecological traits RLQ analysis. Categories are grouped by trait, environmental factors are grouped by variable type, sites are grouped by stream type and taxa are grouped by faunal groups. Codes can be found in Tables 1(traits) and Table 2 (environmental variables)

Plecoptera and most of Ephemeroptera and Trichoptera occupied this right side of the axis, along with *Ancylus* sp., *Austropotamobius* sp., anisopterans as *Cordulegaster* sp. or *Boyeria* sp., the dipteran *Dixa* sp., and water beetles such as Elmidae, *Pomatinus* sp. (Dryopidae) or *Oreodytes* sp. (Dytiscidae). Contrasting ecological characteristic matched the negative part of gradient: common biological traits included high temperature preference, tolerance to low pH and high organic pollution or preference for saline waters and eutrophic systems. They are taxa usually found in potamal sections of streams or even outside of the river system, where they tend to live in marshes, ponds and pools or temporary waters. Preferred microhabitats were microphites, mood or litter substrates and stagnant waters. A number of taxa in Heteroptera, many Hydrophilidae as *Enochrus* sp., *Helochares* sp., *Berosus* sp., or *Paracymus* sp., some dipterans as all Culicidae and Syrphidae, dragonflies and damselflies (*Sympetrum* sp. or *Ischnura* sp.), crustaceans as *Procambarus* sp. and snails as *Potamopyrgus* sp. dominated the left side of the axis. Among the Ephemeroptera, the genus with the lowest score was again *Cloeon* sp.

4. Discussion

4.1. Environmental factors driving community characteristics

Results have shown that a variety of environmental factors was related to the observed community structure and biological/ecological attributes. The most influential variables, i.e., those that showed the highest or lowest values along both biological and ecological RLQ axes were agricultural and natural/forest, riparian forest quality, surficial geology and altitude. There was also a strong spatial influence, with geographical coordinates (and specially longitude) as correlates. Electric conductivity, nitrate, water temperature and discharge also exerted major influences. Among the in-stream habitat variables, those related to substrate size and heterogeneity were the most influential, but to a lesser degree in comparison with the other variables.

Contrastingly, among the variables identified as not being very relevant (those positioned near the centre of the axis) were riparian cover percentage, the in-stream habitat index, the macrophyte cover, the stream order, the catchment area or the oxygen contents

When viewed across relatively large spatial scales, much variation in species distributions can be attributed to patterns of variation within the landscape. Large-scale landscape attributes such as land use, surficial geology, elevation, and hydrology were found by Richards et al. (1996) to have the greatest influence on macroinvertebrate assemblages. However, Richards et al., 1997 found that reach-scale physical features were best related to certain life history and behavioural attributes of macroinvertebrates than catchment-scale variables, suggesting that species traits exhibit strong relationships to local environmental conditions. Our results suggest that a variety of factors acting at different scales and hierarchically organized (for example, agricultural use and percentage of marls are dictating

the conductivity and nitrate content and also influencing the riparian habitat or the substrate) controls the functional organization of communities at our scale of study.

4.2. Taxa traits and environmental attributes

RLQ analysis relates species traits directly to changes in environmental attributes. Thus, major natural environmental variables and anthropogenic alterations important to stream communities were revealed, as well as the biological and ecological traits responding to these properties. We found a highly significant relationship between both biological and ecological traits, expressed by sets of life-history/behavioural/physiological characters and environmental preferences, and the environmental features of the habitats, as measured by the main underlying environmental gradient. In both RLQ analyses, the first ordination axes accounted for very large fractions of the explained variance, indicating the existence of a strong environmental gradient structuring the characteristics of the sites and taxa. This was extremely accentuated in the case of the ecological traits analysis (96 % of variance absorbed by the first axis). The ordination of sites along this axis closely matched our a-priori biotic classification of stream types. *Saline streams* were located in the left extreme of the gradient, while *agricultural streams* and *springs* occupied more central (but still negative) positions (compare Figures 11 and 12). The nature of the environmental factors that dominate this part of the gradient (disturbed land-use, no riparian forests, south-eastern gradient, high nutrient loadings, marly geology, high conductivities, etc.) would indicate an area with very high anthropogenic pressure and so that very threatened ecosystems (Gómez et al., 2005). At the same time, it would reflect a natural disturbance regime typical of semi-arid streams, characterized by extremes of flood and drought, these events being the most important natural disturbance agents in these ecosystems (Fisher & Grimm, 1991). Both factors, along with a high tolerance to salinity control and shape the structure

and functioning of the whole aquatic ecosystem and influence the characteristics of the biota of these characteristics streams locally called "ramblas". (Gómez et al., 2005). This region of the gradient matched some of the biological traits so cited in the literature as providing resilience and/or resistance to disturbances, i.e. small sizes and high number of generations per year (Southwood, 1977, 1988; Townsend and Hildrew, 1994). Other biological features that remove or reduce the impact of environmental fluctuations were the presence of adults known to cope with drought episodes by resistant cells (such as the hydrophilid water beetle *Berosus*), diapause and dormancy as resistance stages and the laying of endophytic eggs. The presence of aquatic adults can also provide with colonization mechanisms after floods or drought disturbances. In this sense, the aquatic adults of Heteroptera and Coleoptera are among the best cyclic colonizers of ephemeral waters and exemplify the adaptations for this lifestyle (Ortega et al., 1991; Wissinger, 1997 and references therein; Velasco et al., 1998; Bilton et al., 2001). The prevalence of ovoviviparity as reproductive strategy was mainly due to the presence of the introduced freshwater snail *Potamopyrgus antipodarum*. This trend have been noted in other works (Doledec et al., 1999; Usseglio-Polatera and Beisel, 2002), where parental care seemed to be more diverse at reference sites where four types of egg deposition predominated, while ovoviviparity and regeneration were more frequent at the frequently disturbed sites. This snail often shows parthenogenetic reproduction, what could also be taken as a factor conferring resilience in disturbed habitats.

The dominance of fliers in the more stressed environments could reflect a prevalence of high immediate dispersal ability to cope with disturbance episodes such as floods and droughts by behavioural adaptations on a per-event basis (Lytle and Poff, 2004).

Nevertheless, some traits usually predicted in more stable and benign conditions were also present in this part of the gradient, i.e. biggest sizes and vertebrate predation. Looking back to the original trait matrix, we observed that the introduced American crayfish *Procambarus clarkii* was the taxa responsible of these characters, mainly in the *agricultural streams*. This species shows characteristics such as opportunistic omnivorous feeding, resistance to pollution and extreme environmental conditions, fast growth and high reproduction potential, it requires productive water systems, etc. which make this species particularly suitable for invading streams affected by cultural eutrophication (Angeler et al., 2001; Gil-Sanchez and Alba-Tercedor, 2002)

Contrastingly, the right side of the gradient (Figures 11 and 12) was mainly occupied by samples from stream sites in semi-pristine conditions, located in the well preserved nature reserves of the humid north-western upper catchment. They corresponded to the *mountain streams* typology. The biological traits highlighted large sizes and less than one reproductive cycle per year. These features have been commonly treated as indicative of relatively stable habitats with a disturbance regime characterized by low frequency and intensity and high predictability (Southwood, 1988; MacArthur and Wilson, 1967; Stearns, 1992; Townsend and Hildrew, 1994). Other characters such as the production of cemented isolated eggs could be justified as a lesser investment in parental care in a more stable environment. Statzner et al. (2001) found that streams having sewage influence had significantly lower abundances of taxa producing isolated cemented eggs in comparison with reference sites, although they did not find a mechanistic explanation to this trend.

Statzner et al. (2001) hypothesize that compared to reference conditions, streams affected by sewage input (i.e. reduced oxygen content of the water) would favour various types of aerial respiration, and abundant land-use in the catchment (i.e. increased erosion and thus

silting of the interstices in the bottom) would disfavour endobenthics; also, the destruction of the riparian forest (i.e. reduced CPOM input to the stream) would disfavour feeding on coarse plant detritus. We found all these trends to occur in our study. The aerial respiration probably was related to low dissolved oxygen levels on account of a mix of factors, i.e. high temperatures and low flows, high nutrient contents or high suspended solids. Nevertheless, oxygen concentrations did not appear to be very relevant in the RLQ analysis, being the sampled streams well oxygenated in most of cases. Aerial respiration is mainly showed by Coleoptera, Heteroptera and Diptera, and probably factors other than oxygen could be favouring this trait in these streams (e.g. temperature and salinity tolerance). We also found a dominance of filters in the mountain streams, almost certainly as a negative response to the low flows characterizing semi-arid streams. Organisms with permanent attachment to the substrate, burrowers and interstitial animals were more abundant in the right part of the graph, coinciding with the *mountain streams*. Usseglio-Polatera and Beisel (2002) also found a similar trend (changes in predominance from swimmer and/or crawler to interstitial or burrower) in a longitudinal characterization of invertebrate communities along the Meuse River. They found as well a change in feeding habits (piercer and scraper to filterer or deposit feeder) that match our results too.

In contrast, the ecological traits featured (from left to right in the RLQ_e axis) saprobity of taxa (poly- to xenosaprobic), salinity tolerance (brackish to freshwaters), trophic status preference (eutrophic to olygotrophic), longitudinal distribution (outside river system to epi-rithral sections), transversal distribution (from marshes and ponds to main channels), current velocity (fast to null), and microhabitat (microphytes to rocky substrates) as 'significant' factors driving the differential ecological features of communities.

4.3 A "habitat templet" for streams in SE Spain

Habitats and species traits can be matched directly, but this match occurs in complicated ways because species traits are expressed in variable combinations in organisms. Consequently, one can always find organisms with unexpected trade-offs of traits in a given habitat that violate the assumptions of the general theoretical model (Williams & Feltmate, 1992; Resh et al., 1994; Townsend & Hildrew, 1994).

The habitat templet originally developed by Greenslade (1983) was applied to lentic waters in the semi-arid regions in Australia (Williams, 1985). The axes of this templet were the extent of predictability (i.e. disturbance regime) and the salinity level. Our results appeared to fit this model, with our RLQ axis being equivalent to the diagonal of the templet (Figure 13). Nevertheless, it is difficult to express the longitudinal axis of the templet in terms of a single variable (namely salinity) due to the variety of confounding factors that correlates along the main gradient in our catchment (altitude and climate, geology and land-use, etc. see Chapter 2). Thus, we opted to call this axis the "adversity axis" (as in the original work by Greenslade), along which natural stressors (i.e. salinity and temporality) are accentuated by human pressures (agricultural land-use and its corollaries: organic enrichment, water diversions and physical channel alterations).

4.4 Biological vs ecological traits

We found an almost perfect correlation between the first RLQ axis obtained using biological traits and the one obtained using the ecological traits, which means that each of the ecological traits was strongly related to one environmental variable measured (and often more than one, e.g. the preference for eutrophic waters could be related to nitrate content, conductivity or agricultural land use) and also that the genus-level ecological information

used from Tachet et al. 2000 reflected in a direct way the measured environmental trends. This matching can also highlight physiological constraints in distribution patterns (see Chown & Gaston, 1999). For example, organisms with aerial respiration can tolerate high
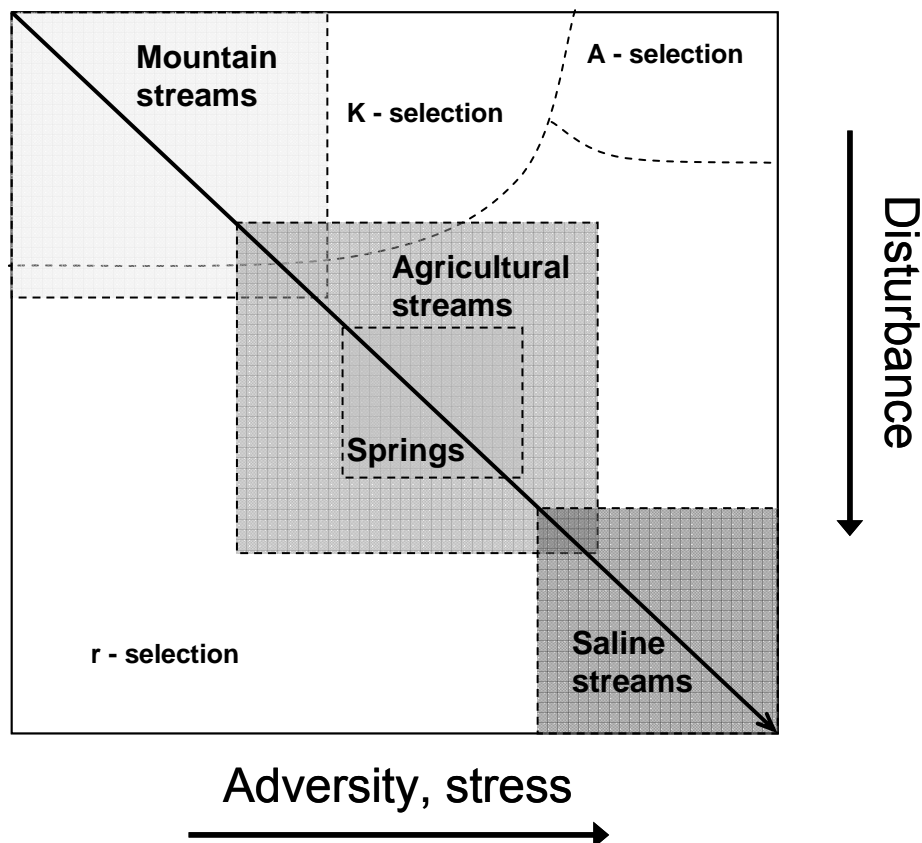
Figure 13. Proposed theoretical habitat templet for streams in the study area. The stream types studied are located along an adversity axis and a disturbance axis. The main selection models acting in each region of the templet are also indicated. The main diagonal corresponds to the obtained first RLQ axes.

salinity and organic pollution and show preference to still waters, while organisms that breathe through tegument show the opposite trends, maybe due to osmotic regulation constraints.

4.5 Future use of species traits as basis for monitoring human impacts

Theory predicts different life-history strategies for different disturbance levels or spatio-temporal heterogeneity (Poff and Ward 1990; Townsend and Hildrew, 1994), and thus different life-history traits can also indicate different intensities of human impact (Dolèdec et al., 1999)

RLQ analysis has been used as an effective tool for identifying species traits that respond to impacts of land use change on different scales. In the initial stage of a monitoring program, it is suitable to select indicator species traits directly linked to specific ecological processes modified by human intervention (Hausner et al., 2003). In regards to human impacts in streams from the Segura river basin, we found changes in land-use (mainly the agricultural use, with the alteration of riparian corridors, water diversions and nutrient enrichment) to have the strongest impact on invertebrate assemblages at the catchment scale. Nevertheless, the confounding effects of geology, altitude and climate precluded any selection of traits responding to environmental impacts.

Mediterranean-type streams and specially those in semi-arid regions are particularly susceptible to water diversion (directly or via groundwater withdrawal) and flow regulation (Gasith and Resh, 1999). Intensive agriculture has proved to be the more destructive human activity (and more than traditional fruit cultivation) in riparian communities from the semi-arid south-east of Spain (Salinas et al. 2000) and the expansion of intensive irrigation crops in Murcia region the last decades have been documented (Martínez-Fernández et al., 2000; 2002 Vidal-Abarca et al., 2004). Moreover, in a recent work Ortega et al. (2004) found that intensive agriculture (expressed as potential nitrogen export) was the main pressure single predictor affecting the ecological integrity of semi-arid wetlands in south-east Spain. Nevertheless, its effects in stream macroinvertebrate communities have

not been addressed properly yet, although semi-arid saline "ramblas" of south-eastern Spain are among the most threatened aquatic ecosystems in Spain and probably in Europe (Abellán et al. 2005; Sánchez-Fernandez et al., 2004; Gomez et al., 2005). Thus, it would be desirable to investigate the effects of this land-use change separately, eliminating those confounding effects as far as possible. Nevertheless, we recognize the difficulty of such a study in our area, at least at a catchment scale study as this.

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Appendix 1. List of macroinvertebrate taxa used in the study. Codes appearing in the figures are included.

Appendix1

Taxonomic unit (Code)	Taxa	Code	Taxonomic unit (Code)	Taxa	Code	Taxonomic unit (Code)	Taxa	Code	Taxonomic unit (Code)	Taxa	Code	Taxonomic unit (Code)	Taxa	Code
Coleoptera (Col)	*Agabus*	Agab	**Heteroptera (Het)**	*Anisops*	Anis	**Trichoptera (Tric)**	*R.(Pararhyacophila)*	pRhy	**Megaloptera (Mega)**	*Sialis*	Sial	**Crustacea (Crus)**	*Echinogammarus*	Echi
	Anacaena	Anac		*Aquarius*	Aqua		*R.(Rhyacophyla)*	Rhy	**Diptera (Dip)**	*Psychodidae NID*	PsycN		*Crangonyx*	Cran
	Aulonogyrus	Aulo		*Gerris*	Gerr		*Hydropsyche*	Hydrps		*Pericoma*	Peri		*Atyaephira*	Atya
	Berosus	Bero		*Hydrometra*	Hydrm		*Cheumatopsyche*	Cheu		*Psychoda*	Psyc		*Austropotamoebius*	Aust
	Bidessus	Bide		*Mesovelia*	Mesov		*Polycentropus*	Poly		*Berdeniella*	Berd		*Procambarus*	Proc
	Cyphon	Cyph		*Micronecta*	Micrn		*Plectrocnemia*	Plect		*Tonnoiriella*	Tonn	**Mollusca (Mol)**	*Pisidium*	Pisi
	Deronectes	Dero		*Microvelia*	Micrv		*Cyrnus*	Cyrn		*Dixa*	Dixa		*Ancylus*	Ancy
	Dryops	Dryo		*Naucoris*	Nauc		*Chimarra*	Chim		*Dixella*	Dixe		*Ferrisia*	Ferr
	Dytiscus	Dyti		*Nepa*	Nepa		*Lype*	Lype		*Culicidae NID*	Culi		*Theodoxus*	Theo
	Elmis	Elmi		*Notonecta*	Noto		*Tinodes*	Tino		*Culex*	Cule		*Lymnaea*	Lymna
	Elodes	Elod		*Plea*	Plea		*Agapetus*	Agap		*Culiseta*	Culis		*Physella*	Phys
	Enochrus	Enoc		*Sigara*	Siga		*Hydroptila*	Hydrpt		*Anopheles*	Anop		*Potamopyrgus*	Potapy
	Esolus	Esol		*Velia*	Veli		*Oxyethira*	Oxye		*Simuliidae NID*	SimuN		*Planorbis*	Plan
	Graptodytes	Grap	**Ephemeroptera (Ephe)**	*Baetis*	Baet		*Stactobia*	Stac		*Simulium*	Simu	**Tricladida (Tricl)**	*Tricladida NID*	Tric
	Gyrinus	Gyri		*Centroptilum*	Cent		*Ithytrichia*	Ithy		*Eusimulium*	Eusi	**Hirudinea (Hirud)**	*Helobdella*	Helob
	Haliplus	Hali		*Cloeon*	Cloe		*Orthotrichia*	Orthot		*Dasyhelea*	Dasy		*Dina*	Dina
	Helochares	Heloc		*Procloeon*	Procl		*Micrasema*	Micra		*Ceratopogoninae*	Cerat	**Oligochaeta (Olig)**	*Oligochaeta*	Olig
	Helophorus	Helop		*Pseudocentroptilum*	Pseuc		*Drusus*	Drus		*Atrychopogon*	Atry		*Eisseniella*	Eiss
	Hydraena	Hydra		*Caenis*	Caen		*Limnephilus*	Limne		*Chironomini*	Chir			
	Hydrochus	Hydrch		*Paraleptophlebia*	Paral		*Potamophylax*	Potamo		*Chironomus*	Chirm			
	Hydrocyphon	Hydrcy		*Habroleptoides*	Habrl		*Halesus*	Hale		*Orthocladiinae*	Orthoc			
	Hydroglyphus	Hydrg		*Habrophlebia*	Habrp		*Stenophylax*	Steno		*Tanypodinae*	Tanyp			
	Hydrophilus	Hydrph		*Chorotherpes*	Chor		*Mesophylax*	Meso		*Tanytarsini*	Tanyt			
	Hydroporus	Hydrpo		*Serratella*	Serr		*Allogamus*	Allo		*Corynoneurinae*	Cory			
	Hygrotus	Hygr		*Torleya*	Torl		*Lasiocephala*	Lasi		*Tipula*	Tipu			
	Hyphidrus	Hyph		*Ephemera*	Ephe		*Ceraclea*	Cera		*Antocha*	Anto			
	Ilybius	Ilyb		*Ecdyonurus*	Ecdy		*Oecetis*	Oece		*Dicranota*	Dicr			
	Laccobius	Laccb		*Heptagenia*	Hept		*Athripsodes*	Athri		*Helius*	Heli			
	Laccophilus	Laccp		*Rithrogena*	Rith		*Setodes*	Seto		*Hexatoma*	Hexa			
	Limnebius	Limnb		*Epeorus*	Epeo		*Mystacides*	Myst		*Pseudolimnophila*	Pseul			
	Limnius	Limni		*Electrogena*	Elec		*Sericostoma*	Seri		*Eloeophyla*	Eloe			
	Meladema	Mela		*Potamanthus*	Potama		*Sericostomatidae*	SeriN		*Paradelphomyia*	Parad			
	Nebrioporus	Nebr	**Odonata (Odo)**	*Anax*	Anax		*Beraeidae NID*	Bera		*Eriopterini*	Erio			
	Normandia	Norm		*Aeshna*	Aesh		*Calamoceras*	Cala		*Hemerodromiinae*	Heme			
	Ochthebius	Ocht		*Boyeria*	Boye	**Plecoptera (Plec)**	*Perla*	Perl		*Clinocerinae*	Clin			
	Orectochilus	Orec		*Sympetrum*	Symp		*Dinocras*	Dino		*Dolichopodidae*	Doli			
	Oreodytes	Oreo		*Crocothemis*	Croc		*Isoperla*	Isop		*Atherix*	Athe			
	Oulimnius	Oulim		*Orthetrum*	Orthe		*Nemoura*	Nemou		*Ibisia*	Ibis			
	Paracymus	Parac		*Cordulegaster*	Cord		*Protonemura*	Prot		*Athrychops*	Athry			
	Peltodytes	Pelt		*Gomphus*	Gomp		*Capnioneura*	Capn		*Tabanidae NID*	TabaN			
	Pomatinus	Poma		*Onychogomphus*	Onyc		*Leuctra*	Leuc		*Tabanus*	Taba			
	Rhantus	Rhan		*Coenagrion*	Coen					*Stratyomyinae NID*	StraN			
	Riolus	Riol		*Ceriagrion cf*	Ceri					*Oxycera*	Oxyc			
	Stenelmis	Stene		*Ischnura*	Isch					*Nemotelus*	Nemot			
	Stictonectes	Sticn		*Pyrrhosoma*	Pyrr					*Stratyomis*	Stra			
	Stictotarsus	Stict		*Lestes*	Lest					*Odontomyia*	Odon			
	Yola	Yola		*Platycnemis*	Platy					*Syrphidae*	Syrp			
				Calopteryx	Calo					*Ephydridae NID*	EphyN			
										Ephydra	Ephy			
										Sciomyzidae	Scio			
										Ilione	Ilio			
										Anthomiidae NID	Anth			
										Limnophora	Limno			
										Chrysopilus	Chry			

Chapter 4

Taxa richness, diversity and functional diversity in macroinvertebrate assemblages from the Segura river basin: natural variations and anthropogenic influences.

Chapter 4. Taxa richness, diversity and functional diversity in macroinvertebrate assemblages from the Segura river basin: Natural variations *vs* anthropogenic influences.

Abstract

Functional diversity, the extent of functional differences among the species in a community, is an important determinant of ecosystem processes and can be used as a measure of ecosystem integrity. We applied a recently described measure of biological diversity that incorporates dissimilarities among taxa. Dissimilarities were defined from biological traits (e.g. life history, morphology, physiology and behaviour) and ecological traits (e.g. microhabitat use and other environmental preferences) of stream invertebrate taxa and the resulting index was considered a surrogate for functional diversity. Samples collected in five different stream types were used to: (i) quantify functional diversity from the biological-ecological traits of aquatic invertebrate taxa, (ii) investigate the relationship between classical diversity measures and functional diversity in the studied streams, (iii) compare taxa richness, diversity indices and functional diversity between contrasting stream typologies, and (iv) determine the main environmental factors affecting both classical and functional diversity measures along the environmental gradient. Comparisons of diversity measures between stream typologies showed significant differences for all the variables, although functional diversity was the only one discriminating between naturally saline streams and regulated-canalized or polluted reaches. Environmental variables explaining diversity trends differed between the different metrics. Concerning taxa richness, the best subset of environmental variables was formed by an only variable, electric conductivity, while elevation, nitrite and ammonium were also identified as explanatory factors. Simpson evenness and functional diversity were both best explained by a two variables subset composed of ammonium and nitrite. Other subsets that showed good correlations with both variables included phosphate and suspended solids. Functional diversity demonstrated

a better performance than taxa richness in detecting impairment in stream macroinvertebrate communities of our region. Moreover, its lack of dependence on different natural factors that clearly affected other diversity measures as taxa richness or Shannon entropy, poses this newly developed diversity index as a good candidate metric in assessing human induced community changes in stream ecosystems.

KEYWORDS: Functional diversity, Biological traits, Ecological traits, anthropogenic influences, semi-arid streams, macroinvertebrates, SE Spain

1. Introduction

The effects of human development on ecosystem properties and functioning have been a central topic in ecology since the beginning of the industrial revolution and the subsequent increases in human disturbance rates. Streams are some of the most studied ecosystems in this context mainly due to their human value for water consumption (among other goods and services) and their traditional use as urban-industrial sewage sinks or their natural character on integrating catchment scale activities and processes. Biodiversity changes caused by human economic activities are one of the main focuses of ecological research worldwide and have been treated almost in every ecosystem type for bioassessment purposes. Moreover, global biodiversity loss already represents an important concern in many political agendas in a planetary context (e.g. the 1992 Rio de Janeiro convention on biological diversity, signed by 177 countries).

There is little consensus on a universal metric for measuring biodiversity, which suggests that different metrics can be used depending on the particular study. The number of taxa (or taxa richness) in a community is by far the most used metric in biodiversity assessment due to its simplicity and its general good correlation with classical diversity indices (Gaston and Spicer, 1998). Nevertheless, as argued by many authors (Izsak and Papp, 2000; Barker, 2002; Petchey and Gaston, 2002; Ricotta, 2004), taxa richness and traditional diversity indices such as the Shannon entropy or the Simpson index summarize the information about the incidence, relative abundance or dominance of taxa within a community or sample without regard to differences between taxa. A disadvantage is that all taxa are taken into account on an equal basis regardless of their biological or ecological characteristics and their ensuing function in the ecosystem. This is not sufficient to assess the diversity of communities (Shimatani, 2001). For example, on a functional feeding group basis, a stream

community composed by ten species of collector-gatherers is not equally diverse as another composed of three shredders, three collectors, one filterer, one predator and two parasitic taxa. A range of diversity indices incorporating species differences or relatedness (mainly taxonomic, phylogenetic or genetic) and relative abundances have lately appeared in response to these and other questions (for examples, Clarke and Warwick, 1998; Shimatani, 2001; Barker, 2002; Mason et al., 2003; Ricotta, 2004).

There are already several methods offered for numerically expressing pairwise difference between species. Among others, Solow et al. (1993) used genetic distances; path lengths in the taxonomic hierarchical classification tree were applied by Warwick and Clarke (1995) and aminoacid differences were used by Shimatani (2001). However, biological attributes or features have been rarely used in calculating taxa relatedness in real communities, and methods for quantifying the functional diversity of communities are scarce (Petchey and Gaston, 2002). There are however some examples of the use of species functional traits to assess taxa similarities incorporated in diversity measures: Champeli and Chessel (2002) used foraging substrates of birds in four mediterranean areas of the world; Pavoine et al. (2004) additionally used some morphometric measures on the same data set, and Bady et al. (2005) used fuzzy coded biological traits (morphological, physiological, functional, phenological, etc.) to assess functional diversity of river macroinvertebrate assemblages through the Euclidean Diversity Index (EDC), an index developed by Champely and Chessel (2002) from the complementary use of the unified theory of diversity of Rao (Rao, 1982) and Euclidean metrics.

Functional diversity is an important component of biodiversity and also an important determinant of ecosystem processes (Tilman, 2000; Díaz and Cabido, 2001; Loreau et al., 2001). It is accepted that if an ecosystem is subject to a range of natural and human-caused

environmental stresses or disturbances, then having a diversity of species that encompass a variety of functional response types would act as insurance in fulfilling ecological processes (Hooper et al., 2005). In this context, an index of general functional diversity should include information about a number of different traits, recognizing that many traits may be important if a number of aspects of ecosystem functioning are to be considered. However, as Petchey and Gaston (2002) pointed out, the higher number of traits used to construct a relatedness matrix, the more similar will appear those taxa, decreasing the importance of species identity not because all species are very similar, but because species are similarly different.

One of the problems concerning environmental assessment using biodiversity measures is the natural spatio-temporal variability of ecological communities. Thus, to differentiate these natural dynamics from those arising from human disturbance (by proper experimental designs or by searching useful indicators) represent a challenge for basic and applied ecological research. Taxa richness usually shows wide geographical variations in a range of scales. Altitude, latitude, climate, geology, and more local habitat features are among the well known factors influencing species richness and other community properties in stream ecosystems. However, functional diversity responses to these natural changes are less known, although some functional attribute metrics (namely the number or relative abundance of functional feeding guilds) have been used in classical bioassessment programs (Karr and Chu, 1999; 2000; Barbour et al., 1999). Nevertheless, assigning taxa to particular functional groups is always controversial and depends on the method used (Mason et al., 2003) much more when higher taxonomic levels are considered.

On the other hand, ecosystems confronted to particular human disturbances or to certain degree of stress (intermediate disturbance levels) theoretically can be affected in their

functional structure while maintaining the same (and even greater) richness values than their unaffected counterparts. Furthermore, classical diversity measures and particularly richness are known to have high sensitivity to sample size and effort (Magurran, 1988; Gotelli and Colwell, 2001), and Bady et al. (2005) demonstrated for large European rivers a better performance of functional diversity over genus richness (less sensitivity to sampling effort and more stability in space and time). Additionally, rare species are difficult to estimate and may disproportionately influence species richness (Gaston, 1994).

The landscape in south-eastern Spain ranges from Mediterranean conifer forests in mountain ranges along major nature reserves in the NW, to arid and semi-arid shrublands further south-east. This gradient in altitude and climate is coupled with a human development gradient. The river network has low populated forested headwaters, intermediately populated agricultural midlands with intense flow regulation, and densely populated cities in the lowlands. Natural salinity gradients are also well documented in the Segura River basin (Millán et al., 1988; 1996; Vidal-Abarca et al., 1992; Moreno et al., 2001; Mellado et al., 2002). Some of the tributaries flow across salt-rich rocks such as sedimentary Miocene marls rich in sodium chloride and gypsum and therefore their salinity is naturally high (Moreno et al., 1997; 2001), sometimes showing marked differences in biotic composition compared to their freshwater counterparts in the same region (Moreno et al. 1997; 2001; Bonada et al. 2004, Vivas et al., 2004; Prat et al., submitted).

In this paper we use aquatic invertebrate samples collected in five different stream types previously defined by their community structure and environmental features to: 1) quantify functional diversity from the biological-ecological traits of aquatic invertebrate taxa, 2) investigate the relationship between classical diversity measures and functional diversity in the studied streams, 3) compare taxa richness, diversity indices and functional diversity

between contrasting stream typologies, and 4) determine the main environmental factors affecting both classical and functional diversity measures along the environmental gradient. We hypothesize that functional diversity (as a measure of the integrity of the ecosystem) would be less affected by natural variations in environmental factors than taxa richness, and hence would prove its value as a bioassessment tool in evaluating human impacts in stream ecosystems.

2. Methods

2.1 Study area

The Segura River basin is located in the south east of the Iberian Peninsula. It flows from the NW to the SE and drains a basin of 14432 km^2. The climate ranges from humid in the mountains of the Northwest to semiarid elsewhere. The lithology of the plains is characterized by the predominance of limestone and Miocene and Keuper marls with some volcanic areas, whereas calcites and dolomites dominate the mountain headwaters. Quaternary deposits (clay, sand and gravel) form the river alluvia and cover rivers surrounding areas as a thin layer. More detailed information about the study area and the sampling sites and streams involved in this chapter can be found elsewhere (Robles et al., 2004; Mellado et al., 2002) as well as in previous chapters.

2.2 Biological and environmental data

In this study we used data collected during two different sampling occasions, one data set collected in 1987 (the "lucdeme" survey, see Chapter 2) and another data set from 1999-2001 (the "Seneca" survey, see chapters 1 and 3). Sampling procedures were similar in both occasions, although the sorting methods differed.

Concerning the 1999-2001 part of the data set, 104 macroinvertebrate samples were taken from 16 streams in 7 occasions (see Chapters 1 and 3 for a more detailed description of the sampling procedures). One single multi-habitat kick-sample was taken in each sampling occasion. Macroinvertebrates were live-sorted in the field. The sampling went on until no new taxa (at family level) were found in the field. Invertebrates were identified in the laboratory, usually to genus, except for some dipterans that were identified to family, subfamily or tribe, Hirudinea (identified to family), Hydracarina, Tricladida and Oligochaeta. A total of 208 taxa were used in this study.

Respecting the 1987 survey, another composite multi-habitat kick-sample was taken in each sampling occasion. In this case, the entire sample was preserved in 70% ethanol and returned to the laboratory for subsequent sorting and identification. All organisms were sorted and identified to the lower taxonomic level that was achievable (usually genus). For data analysis we aggregated identifications to a genus level for consistency. Here some groups were also identified to higher taxonomic categories such as family (Diptera and certain Trichoptera), subfamily or tribe (Diptera: Chironomidae). Nematoda, Hydracarina and Oligochaeta were not identified further. We averaged the 4 seasonal abundance records to summarize data in an annual data set consisting of 86 stream sites and 176 taxa.

A number of different environmental variables were measured and/or calculated for both the sampling campaigns (see Chapters 2 and 3) but for this study we could only take into account those twelve variables measured or calculated for both surveys: i.e. percentage natural area of sub-catchment (calculated using GIS hydrological data analysis and CORINE land cover, CEC, 1993), water temperature, pH, dissolved oxygen, electric

conductivity at 25ºC, total suspended solids, main nutrients (P-phosphate, N-nitrate, N-nitrite and N-ammonium), elevation and geographical longitude.

2.3 Biological and ecological traits of invertebrates

We used 62 categories of 11 biological traits and 50 categories of 10 ecological traits to describe the functional diversity of assemblages. A complete list with the traits and categories used is shown in Table 1 of Chapter 3. The selection of traits and their categories was dictated by the available information. The information used here was that in Tachet et al. (2000), in which an affinity score is assigned to each genus for each trait category using a fuzzy coding approach (Chevenet et al., 1994). This trait database described the overall European affinity of each genus to each trait category (Statzner et al., 2004). To give the same weight to each taxa and each biological trait in further analyses, affinity scores were standardized so that their sum for a given taxa and a given trait equaled 1. For some taxa identified at coarser levels of taxonomic resolution, standardized affinity scores were calculated by summing the affinity scores of the genera belonging to this taxonomic group and known from our region and re-scaling the results to a 1-5 scale. The opposite case was also present, for example, when we identified a genus (mostly dipterans) that was not treated at this level in Tachet et al. (2000) but at a coarser one (usually subfamily), we assigned the family or subfamily affinities for that genus. Overall, 209 taxa were described completely in the trait database of the 1999 study, and 176 in the 1987 one.

2.4 Functional diversity assessment

We assessed functional diversity of our invertebrate assemblages using the index developed by Champely and Chessel (2002). This index can be used to take dissimilarities among taxa into account based on their traits.

Consider a $N = [n_{ij}]$ community described by the occurrence of J taxa. Table $P = [p_{ij}]$ (with

$p_{ij} = n_{ij} / \sum_{j=1}^{J} n_{ij}$) contains the frequencies of each taxon in each sample. Matrix $D = [d_{ij}]$

quantifies the distance between the ith taxon and the jth taxon.

Because of the fuzzy coding structure of the trait data, similar to tables that contain allele frequencies (Bady et al., 2005), we computed the dissimilarity among genera for each trait using the Edwards distance, a measure currently used for evaluating genetic distances among loci within a population (Edwards, 1971):

$$d_{ij} = \sqrt{1 - \sum_{k=1}^{h} \sqrt{q_{ik}} \sqrt{q_{jk}}}$$

where q_{ik} (or q_{jk}) is the frequency of the trait category k ($1 \leq k \leq h$) for the taxa i (or j).

We calculated a dissimilarity matrix for each of the 21 traits to provide a full description of the functional diversity of invertebrate genera. The 21 individual matrices were combined through their quadratic mean (Hartl and Clark, 1989) to derive a global dissimilarity matrix of biological traits:

$$D_t = \sqrt{\frac{1}{t} \sum_{s=1}^{t} D_s^2} \qquad (1)$$

where t is the number of traits (in our case 21). From equation 1, we computed our functional diversity index (FDI) for sample s as:

$$\text{FDI}_s = \frac{1}{2} \sum_{j_1=1}^{J} \sum_{j_2=1}^{J} p_{sj_1} p_{sj_2} (d_t)^2_{j_1 j_2} \qquad (2)$$

Functional diversity (FDI) was computed using the *R* software (*R* Development Core Team, 2004)

2.5. Statistical analyses

2.5.1. Grouping of samples and diversity trends

Non metric multiple dimensional scaling (MDS) analysis was applied to the sample similarity matrix (on previously standardized variables and using normalized Euclidian distance) resulting from the environmental data matrix (consisting of the twelve variables measured in both sampling campaigns and 171 samples –some of the original were deleted due to missing data in some of the variables). A total of ten random restarts were conducted and the minimum stress two dimensional solution was selected and plotted. Stress value was recorded. Overall differences between the identified groups were tested by means of analysis of similarities (ANOSIM, Clarke, 1993) with 999 permutations. The *PRIMER* 5 statistical package was used for the MDS ordination and ANOSIM tests (Clarke and Gorley 2001)

Later, we examine pairwise differences in diversity measures between stream types by non parametric Kruskal-Wallis tests and post-hoc Dunn's tests (Zar, 1984) since normality and homocedasticity assumptions could not be met. The statistical package *SigmaStat 3* was used for these analyses.

2.5.2. Functional diversity and diversity measures relationships.

The relation between the FDI and taxa richness, Shannon entropy and Simpson evenness was investigated graphically and then fitted to a linear or logarithmic model if appropriate by means of simple linear regression or fixed non linear regression techniques. Samples

were plotted classified by the stream typology resulting from the MDS to reveal possible trends in their positioning on each bivariate space. The program *Statistica 6* (Statsoft, 2001) was used for regressions and graphics.

2.5.3. Environmental variables and diversity measures relations

The possible relation between the different diversity measures (taxa richness, Shannon entropy, Simpson evenness and the FDI) and the environmental variables measured was assessed by the *BIOENV* procedure (Clarke and Ainsworth, 1993). In this procedure, first, a Euclidean distance matrix was calculated for each diversity measure. Then, a battery of Euclidean distance matrices was constructed using one up to five environmental variables previously log-transformed and standardized. Correlations (Spearman) between the first matrix and all the others were performed. The environmental variables subset that resulted in a distance matrix showing the best correlation with the first matrix was selected as the most explicative for each diversity measure. In order to test for the significance of the relationships, randomization tests (999 permutations) were performed with the *RELATE* procedure (Clarke, 1993). The *BIOENV* and *RELATE* procedures were performed with the *PRIMER 5* package (Clarke and Gorley, 2001).

3. Results

Two outliers (highly polluted sites) were deleted from the original data set for clarity. The two dimensional MDS plot using the 12 environmental variables resulted in a very similar grouping of samples to other ordinations previously showed in other works (chapters 1, 2 and 3) so sample grouping was not modified for consistency (Figure 1). A very low stress value of 0.08 was obtained, indicating a very good correspondence between the original sample distances and those obtained for the two dimensional MDS space. ANOSIM tests

revealed significant pair-wise differences between all the groups except for groups 1 and 4 (p=0.001). Briefly, group 1 was composed of some spring-fed stream reaches at low altitudes. Group 2 was mainly formed by lowland reaches of the main course of the Segura river, with obvious signs of degradation due to pollution and channeling. Group 3 sites were middle reaches of the two principal rivers of the study area, highly regulated and sometimes canalized. Group 4 was composed of medium altitude reaches belonging to different tributaries located in extensive agricultural areas from the middle part of the Segura river basin. Group 5 sites were medium to high altitude stream reaches located in
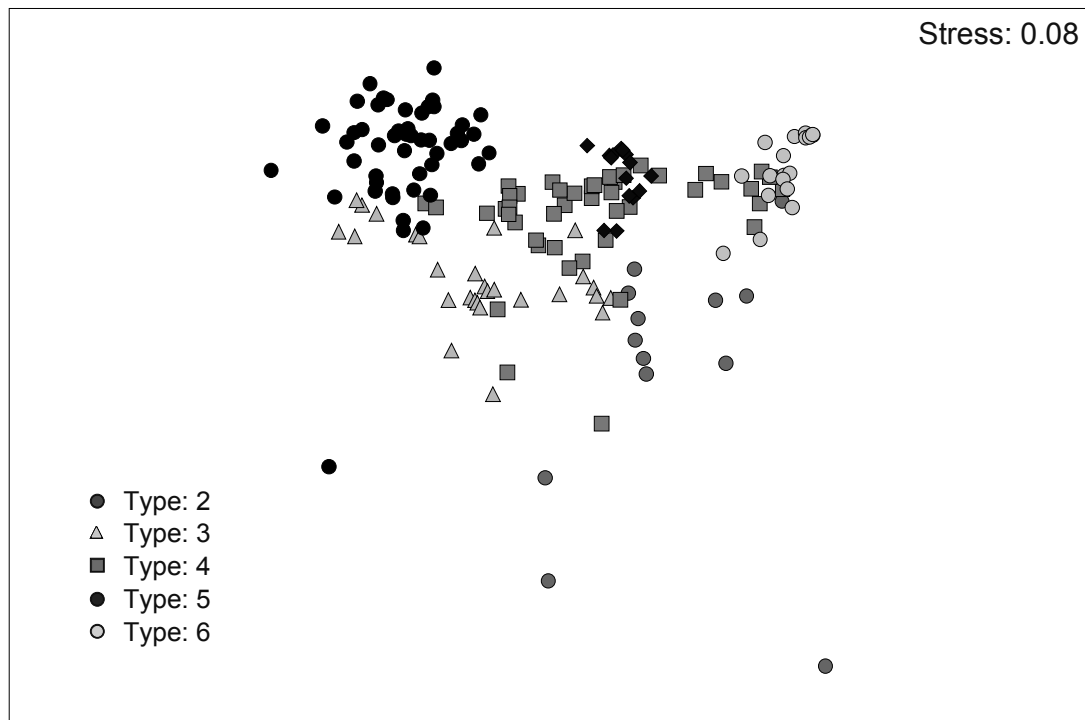

Figure 1. Non metric multiple dimensional scaling (MDS) ordination plot of samples, based on the 12 environmental variables. Samples are identified according to previous classifications based on macroinvertebrate communities (see chapters 1, 2 and 3): Type 2: Lowland polluted sites; Type 3: Regulated middle reaches; Type 4: Agricultural tributaries; Type 5: Mountainous streams; Type 6: Semi-arid saline streams.

mountainous conifer-forested areas inside or near to a nature reserve, in the humid NW part of the basin. Finally group 6 was composed of low altitude, naturally saline streams in semi-arid areas of the south-eastern part of the catchment. For a more concise description

of the stream groups see Chapter 1 (based on part of the 1999-2001 survey, where group MS –mountain streams- correspond to group 5 here; group AS –agricultural streams- corresponds to group 4- and group SS –saline streams corresponds to group 6), Chapter 3 (based on the same period and with the same notation as Chapter 1) and Chapter 2 (based on the 1987 sampling period, where groups 2 and 3 were described, and 4 and 5 were present too –with the same notation as in this paper- are described for the 1987 period).

Comparisons of diversity measures between stream typologies showed significant differences for all the variables (Kruskal-Wallis tests $P<0.001$). Group 1 (spring sites) was not tested for brevity. Mountain streams (Group 5) always reached the maximal diversity values except for functional diversity, higher in agricultural tributaries (Group 4). The lowest values for all the variables were found in lowland polluted sites (group 2) followed by regulated river reaches (group 3). Saline streams (group 6) showed a curious pattern, with low richness values but moderately high Shannon diversity, evenness and functional diversity. Pairwise comparisons (Dunn's tests significant at $P<0.05$) were as follow: taxa richness was significantly higher in groups 5 and 4 (mountain streams and agricultural tributaries). Groups 2, 3 and 6, with lower richness values did not show any significant pairwise difference. The Shannon entropy index was also higher in groups 4 and 5. However, there were also significant differences between groups 6 (saline streams) and 2 (polluted lowland sites), whereas groups 4 and 6 did not differ. The Simpson evenness index and the functional diversity threw up similar trends. Groups 4, 5 and 6 showed higher values and groups 2 and 3 the lowest. However, Simpson evenness did not differ between groups 3 and 6 and did differ between groups 5 and 6. On the contrary, functional diversity was consistently higher in groups 4, 5 and 6 and lower in groups 2 and 3. Within group data dispersion was lower in groups 2 and 3 for taxa richness but much higher for

evenness and functional diversity. Groups 4 and 5 showed the opposite trend, with high variability in taxa richness and low variability in evenness or FDI.

The relationship between taxa richness and functional diversity showed a saturated relationship that could be fitted to a logarithmic function with an adjusted R^2 of 0.51 (Figure 3). There was considerable scatter of samples (for a given level of richness) along the functional diversity axis. Samples having the lowest functional diversities (usually
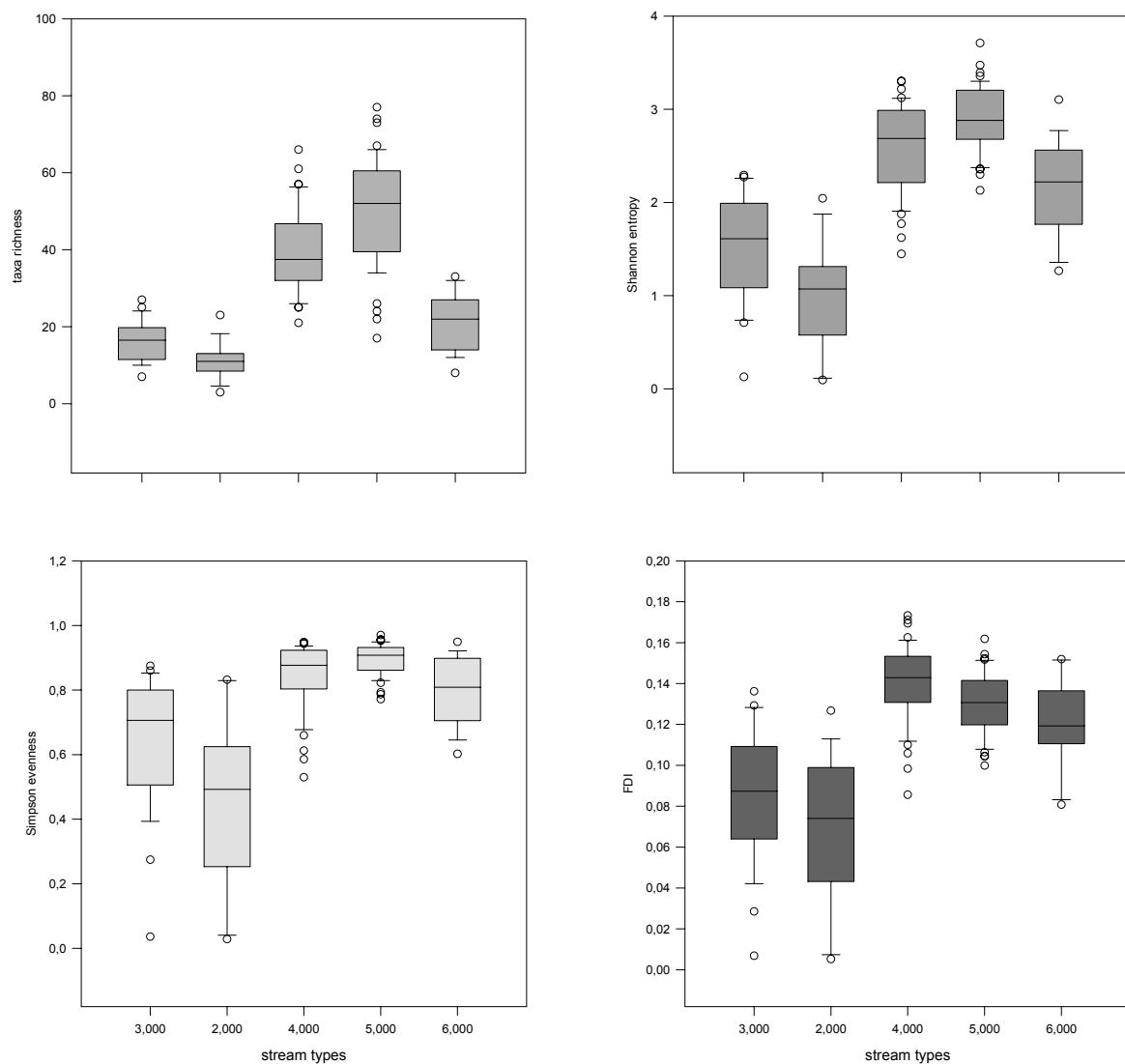

Figure 2. Box plots of the different diversity measures grouped by the main stream typologies. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles.

belonging to groups 2 and 3) tended to present also low richness values, and the opposite trend was also true, with samples from groups 4 and 5. Meanwhile, samples presenting intermediate functional diversities (the region between the dashed lines in Fig. 3) showed a very wide range of richness values. Shannon entropy and Simpson evenness relationships with FDI performed intermediately between the two situations.
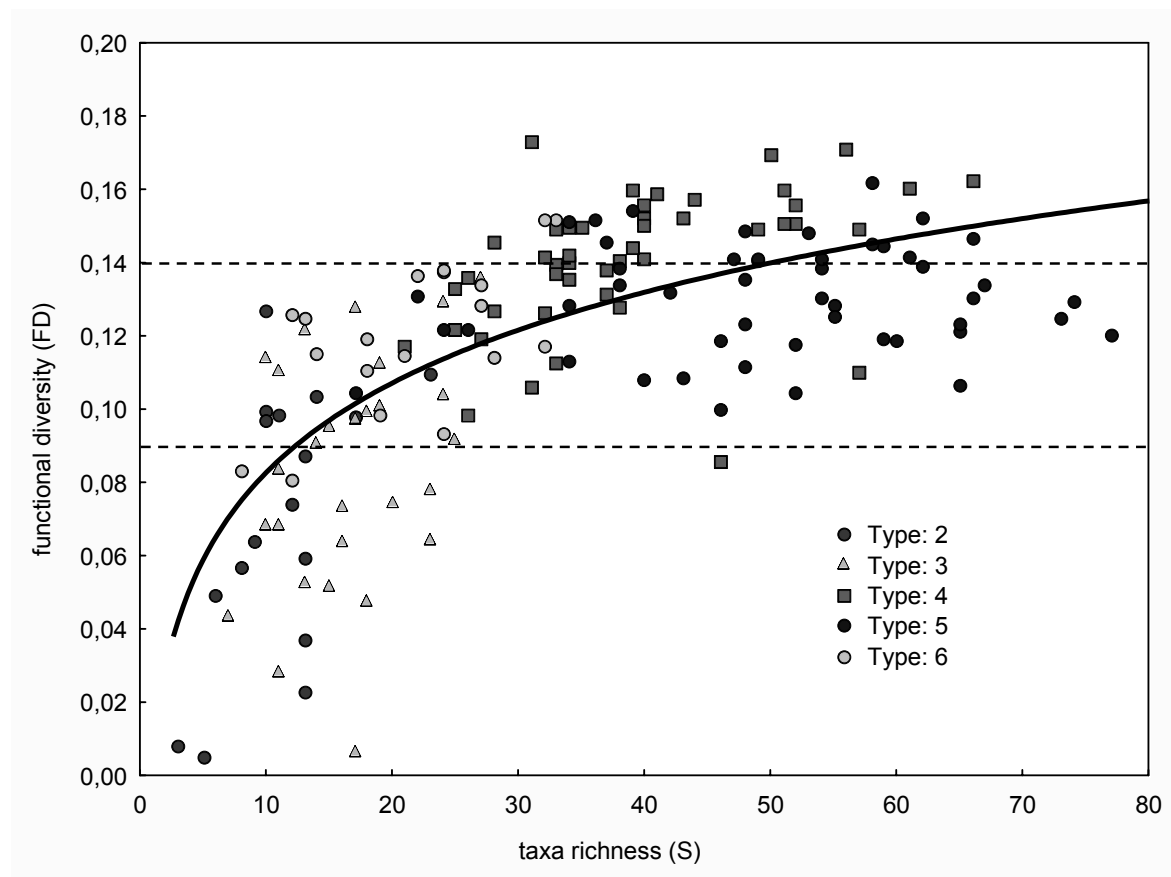

Figure 3. The relationship between taxa richness and functional diversity. The fitted line (fixed non-linear regression) shows a logarithmic function (adjusted $R^2 = 0.51$).

The relationship between Shannon entropy and functional diversity (Figure 4) showed a linear relationship that could be fitted linearly with an adjusted R^2 of 0.71. The relationship

between Simpson evenness and functional diversity (Figure 5) showed also a very clear

linear relationship that could be fitted with an adjusted R^2 of 0.75.

Environmental variables explaining diversity trends differed between the different metrics

(Table 1) Concerning taxa richness, the best subset of environmental variables was formed

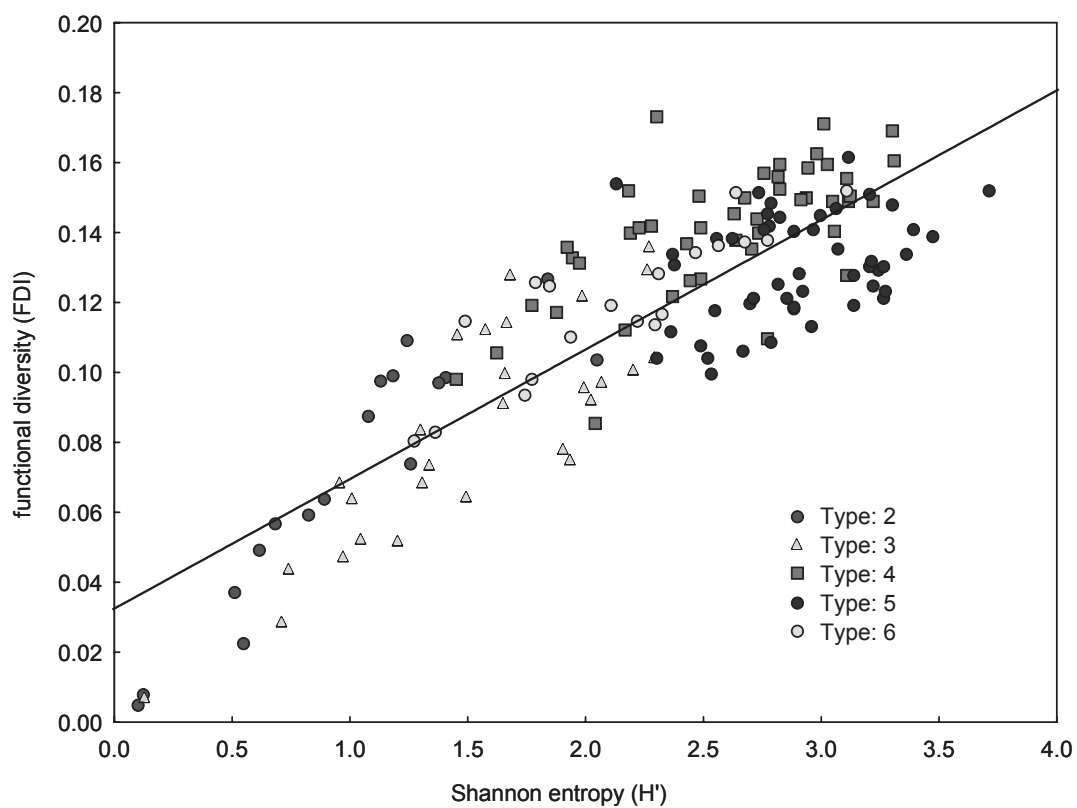by an only variable, electric conductivity, with a Spearman correlation of 0.378. The other

Figure 4. The relationship between Shannon entropy and functional diversity. The fitted line (simple linear regression) shows a linear regression function (adjusted $R^2 = 0.71$).

subsets identified always presented electric conductivity, while elevation, nitrite and

ammonium were also present. Shannon entropy was best explained by the combination of

four variables, altitude, suspended solids, ammonium and nitrite, with a correlation of

0.309. Phosphate was the only one variable that newly appeared in the other three better

subsets. Simpson evenness and functional diversity both were best explained (Spearman correlations of 0.361 and 0.322 respectively) by a two variables subset composed of ammonium and nitrite (Table 1). Other subsets that showed good correlations with both variables included phosphate and suspended solids. All best subsets correlations were significant (RELATE procedure, 999 permutations, p= 0.001).
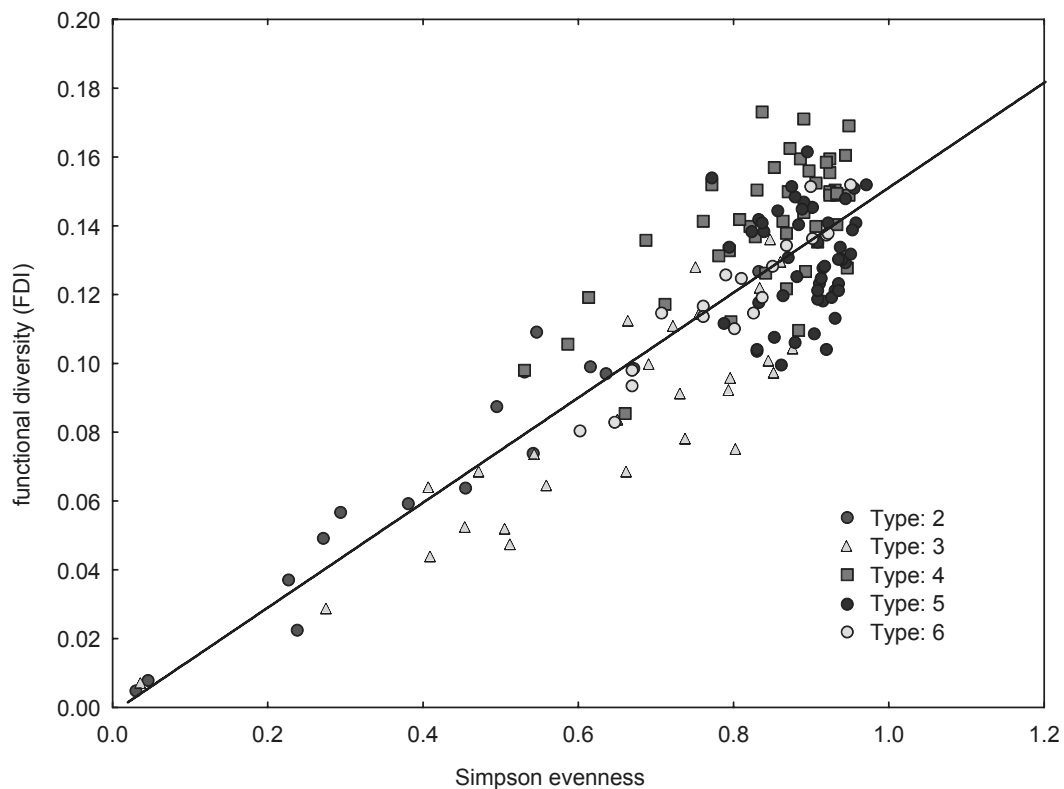

Figure 5. The relationship between Simpson evenness and functional diversity. The fitted line (simple linear regression) shows a linear regression function (adjusted $R^2 = 0.75$).

4. Discussion

IUCN (1980) interpreted the value of biodiversity as its *option value* for the future: the greater the amount of biodiversity we conserve today, the greater the possibilities for future biodiversity because of the diverse genetic resource needed to ensure continued evolution in a changing and uncertain world. Biodiversity can then be expressed as the number of

species plus the variety of activities each species undergoes during its life plus the non phenotypic expression on its genome (Erwin, 1991). Thus, the more of these aspects one can integrate in a diversity measurement, the more precise and informative will result.

Taxonomic or phylogenetic diversity indices (those incorporating taxonomic differences among taxa) have been applied to cover a range of ecological applications, from the selection of species and areas for conservation (Vane-Wright et al., 1991; Faith, 1992; Polaski et al., 2001; Rodrigues and Gaston, 2002) to the biomonitoring of different human impacts or management scenarios (Warwick and Clarke, 1995, Shimatani, 2001; Mouillot et al., 2005). However, Clarke and Warwick (1998) pointed out that there had been little carry over of phylogenetic diversity and related diversity measures into the area of environmental monitoring and assessment.

Table 1. Best 4 subsets of explanatory variables identified by the BIOENV analysis for each diversity measure. The spearman rank correlation coefficient is showed for each subset. Variables in bold represent the best subsets overall.

Variable		Best subset	Other subsets		
Richness (S)	Correlation Selections	0.378 **Conductivity**	0.356 Conductivity Nitrite	0.330 Conductivity Altitude	0.326 Conductivity Ammonium
Shannon (H')	Correlation Selections	0.309 **Suspended-solids Altitude Ammonium Nitrite**	0.309 Suspended-solids Altitude Ammonium Nitrite Phosphate	0.307 Ammonium Nitrite	0.305 Suspended-solids Nitrite Altitude
Simpson (1-λ)	Correlation Selections	0.361 **Ammonium Nitrite**	0.348 Phosphate Ammonium Nitrite	0.342 Nitrite	0.335 Phosphate Nitrite
FDI	Correlation Selections	0.322 **Ammonium Nitrite**	0.311 Phosphate Ammonium Nitrite	0.308 Suspended-solids Ammonium Nitrite	0.308 Suspended-solids Phosphate Ammonium Nitrite

Although many authors have underlined the direct relationships between taxonomic diversity and functional or attribute diversity (as Warwick and Clarke noted for marine nematodes taxonomic and trophic diversity), there are not many examples of using functional features directly to calculate diversity, probably because of difficulties in quantifying pairwise differences between species (Shimatani, 2001). Recent developments have been done in this context. For example, Petchey and Gaston (2002) generalized the use of the phylogenetic diversity index (*PD*, i.e. the total branch length of a known phylogenetic tree represented in a community; Faith, 1992, 1994) to characterize the functional diversity of a community by using cluster classifications obtained from any other functional attributes matrix of the species.

Because a "complete" set of all features for a set of species will never be observed directly, inference of relative contributions of different species to overall feature-diversity will have to be made using some surrogate. We have applied a recently described measure of functional diversity that incorporates dissimilarities defined from biological traits (e.g. life history, morphology, physiology and behaviour) as well as ecological traits (e.g. microhabitat use, thermal tolerance, longitudinal optimum, pollution tolerance, etc.) of stream invertebrates (Table 1, Chapter 3).

Our results showed a clear discrimination among different stream types based on environmental variables, in good consonance with previous biotic classifications based on macroinvertebrates. Functional diversity of macroinvertebrate communities clearly discriminated between the most human-impacted sites (groups 2 and 3) and the other stream typologies including the naturally saline streams of group 6. On the contrary, taxa richness did not discriminated between groups 2, 3 and 6, what was clearly reflecting the influence of natural environmental variations (mainly geology-dependent salinity and

altitude) over this parameter. Salinity is a factor affecting community composition and richness in streams and lakes (Leland and Fend, 1998; Moreno et al., 1997; Halse et al., 2002). The natural salinity gradient that exist in aquatic ecosystems in our study area (Millán et al., 1988; 1996; Mellado et al., 2002; see also preceding chapters) as well as in the Iberian Mediterranean basin (Vivas et al., 2004) and other parts of the Iberian peninsula (Gallardo-Mayenco, 1994) sometimes makes it difficult to apply classical biomonitoring metrics as species richness to assess human disturbances in these ecosystems. Hence, it seems necessary to find other indicators of ecosystem properties or functioning which being readily influenced by pollution and other human caused stresses, are not so influenced by natural environmental variations such as salinity. In our study we found that the functional diversity index applied (as well as the Simpson evenness index) fulfilled this condition, although specific studies such as before and after control-impact (BACI) experiments in saline streams are essential to better test its utility as a biomonitoring tool.

In a similar manner and reinforcing this idea, our results showed that variations in electric conductivity and to a lesser degree elevation were among the primarily drivers of the variability in taxa richness, along with two typical indicators of stream pollution as ammonium or nitrite. However, only these two pollution indicators along with phosphate and total suspended solids were the major determinants of changes in functional diversity as well as in Simpson evenness. The increasing trend observed in taxa richness with elevation in our study area can be explained simply by climatic factors, as temperature, precipitation and altitude are strongly correlated in our region as well as in many temperate and arid habitats (see chapter 2; Brown, 1995; Gaston, 2000). A similar trend in species richness has been reported from other areas, with peak richness values located at mid elevations (Rahbek, 1995; 2005). However, other confounding factors coupled with the NW to SE altitudinal gradient such as land use intensity –human impact- (as observed in

other studies, e.g. Tate and Heiny, 1995; see also chapter 3) or the geology-salinity gradient (see chapter 3) are probably affecting this trend, while historical factors such as isolation of mountainous habitats could also be involved (Lomolino, 2001).

To separate the effects of natural local and/or regional environmental factors from those derived from human activities is one of the main methodological problems in studying ecosystems in our study area (Chapter 2). The differential response of the variables studied offers good opportunities to disentangle these inter-correlation problems.

The saturating relationship observed between taxa richness and functional diversity and the positioning of the different stream groups on it, suggests a functional diversity threshold from which separate heavily impacted communities from those better preserved (located somewhere in between dotted lines on figure 4). However, a more precise classification of sites in terms of human impact, having reference or semi-pristine sites and differentially impacted sites in different stream ecotypes would be necessary to precisely define this "threshold". This possible lack of precise definition or grouping according to human induced stress is probably responsible of the high within-type dispersion observed in functional diversity (Figure 3).

The very similar behavior observed for functional diversity and the Simpson evenness index can be explained by the fact that both indices are greatly influenced by the relative abundances of the main taxa. Moreover, using a number of different traits to calculate the functional diversity can result in a fairly similar distance k between any pair of different taxa (if we have a lot of traits, is easy to be distinct in some of them and similar in others).

If this is true, it is easy to demonstrate that the FDI (Eqn. 2) approximates to the Simpson index, $D = 1 - \sum_{j=1}^{J} p_j^2$, multiplied by a constant:

$$FDI = \frac{1}{2} D\, k^2$$

This could be the case in our study. However, we tried to test this assumption by using only the first four traits (see Table 1, Chapter 3) and the results were very similar to that obtained with the total trait matrix (the correlation between the two functional diversity measures was 0.98). These results are quite ambiguous and point out the redundancy between these two metrics. Thus we acknowledge the need of more basic research on the mathematical properties of this diversity measure and its dependence on the distance measures used.

In conclusion, the functional diversity index has demonstrated to better perform in detecting impairment in stream macroinvertebrate communities of our region than taxa richness. Its lack of dependence on different natural factors that clearly affected other diversity measures as taxa richness or Shannon entropy, poses this newly developed diversity index as a good candidate metric in assessing human induced community changes in stream ecosystems. Furthermore, its independence from sampling effort and rare taxa (Bady et al., 2005) make it a more reliable measure, while the fact that no extra field data are required to its calculation makes the functional diversity index an easily applicable metric to monitor the integrity of macroinvertebrate communities in streams and other environments.

On the other hand, function-related measures of biological integrity may be more ecologically relevant in that they reflect in-stream processes, and changes may indicate that

community and ecosystem resistance to stress has been exceeded (Huryn et al., 2002; Woodcock and Huryn, 2004). Nevertheless, the temporal and local (reach scale) spatial variability of the functional diversity indices must be addressed. In this sense, Bady et al. (2005) found their functional diversity measure to be more stable temporally and spatially than richness.

Besides the solid theoretical foundations over which functional diversity is based, offers the possibility of modifying the trait spectrum according to the objectives of a study, by choosing the particular features a priori involved in the specific change or process that we want to evaluate. For example, if we want to test the effect of siltation in a stream macroinvertebrate community, one could chose as functional response traits the microhabitat distribution (clogging of interstices), the respiration method (aerial would become dominant) and/or the feeding habits (filterers would result disadvantaged). Another possibility is the finding of the best trait subset that is involved in any observed process, offering a more mechanistic understanding of ecological processes and establishing a framework to develop new hypotheses to test experimentally.

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Conclusiones

Conclusions

Conclusiones generales

1. No fueron observadas tendencias estacionales que pudieran oscurecer la agrupación de muestras de distintos tipos de ríos. Tampoco se observaron diferencias estacionales para ninguna de las variables consideradas. Estos resultados son indicativos de una persistencia temporal de los patrones de la comunidad.

2. Los resultados multivariantes sobre la eficiencia y funcionamiento de la metodología de muestreo *in vivo* son mejores que aquéllos obtenidos mediante el método de submuestreo tipo *fixed-count*. Las diferencias en las métricas de la comunidad también ilustraron este hecho. No encontramos ningún sesgo en relación al tamaño de los organismos.

3. La identificación a nivel de familia proporciona resultados comparables al nivel de género a una escala de estudio de cuenca, mientras que la identificación de géneros descubriría diferencias más sutiles dentro de las comunidades. Los datos de presencia-ausencia pueden explicar la mayoría de variación en la estructura de la comunidad.

4. Se han identificado seis tipos de ríos en la Cuenca del Río Segura, con diferentes comunidades de macroinvertebrados y táxones indicadores y factores ambientales distintivos: ríos de montaña, ríos agrícolas, arroyos salinos, cursos medios y cursos bajos contaminados.

5. La salinidad y la contaminación son los factores clave que gobiernan los modelos de distribución de macroinvertebrados a gran escala en la Cuenca del Segura. Este gradiente principal se correlaciona con el gradiente longitudinal de la cuenca, desde tramos o ríos de agua dulce a elevada altitud, prístinos, en las cabeceras húmedas del NO a los tramos más bajos y contaminados del SE más árido.

6. Las variables más influyentes para la estructura de la comunidad normalmente operan a escala regional, viéndose afectadas por los patrones climáticos y/o geológicos, o de usos del territorio.

7. Se encontró una relación altamente significativa entre los rasgos biológicos y ecológicos de los macroinvertebrados y las características ambientales de los hábitats que ocupan.

8. Las variables más influyentes fueron el uso del suelo, la calidad de las riberas, la geología y la altitud. Se observó también una fuerte influencia espacial. La conductividad eléctrica, el nitrato, la temperatura del agua y el caudal también ejercieron importantes influencias. Entre las variables de hábitat fluvial, aquéllas relacionadas con el tamaño del sustrato y su heterogeneidad fueron las más influyentes, pero en menor grado comparadas con las otras variables.

9. Nuestros resultados sugieren que una gran variedad de factores que actúan a diferentes escalas, organizadas jerárquicamente, controlan la organización funcional de las comunidades a nuestra escala de estudio.

10. El índice de diversidad funcional ha demostrado un mejor funcionamiento para la detección de impactos en las comunidades de macroinvertebrados de ríos y arroyos en nuestra región que la riqueza de taxa. Su falta de dependencia de diferentes factores naturales que afectan claramente otras medidas de diversidad, hacen de este índice un buen candidato para la evaluación de los cambios provocados por el hombre en los ecosistemas de ríos y arroyos.

11. Las sólidas bases teóricas en que descansa el concepto de diversidad funcional, ofertan la posibilidad de modificar el espectro de rasgos según los objetivos de un estudio, escogiendo aquellos rasgos a priori involucrados en el cambio o proceso específico que queremos evaluar.

General conclusions

1. We did not find any pattern of seasonal trends that could have obscured the grouping of samples from the same stream typology. We did not observe seasonal differences for any of the variables considered. These results are indicative of a temporal persistence in community patterns.

2. Multivariate results on the applicability and performance of the live-sorting methodology are better than those achieved with a fixed count subsampling method. Differences in community metrics also illustrated this fact. We did not find any bias concerning the size of the organisms.

3. Family level identification provides comparable results to the genus level at a broader environmental scale, while genus identification performs better in detecting subtle differences within communities. Presence-absence data can explain the majority of variation in community structure.

4. Six types of streams have been identified in the Segura River Basin, with contrasting macroinvertebrate communities and indicator taxa and distinctive environmental factors: mountain streams, agricultural streams, saline streams, middle courses and lower contaminated reaches.

5. Salinity and pollution are the keystone factors governing broad-scale macroinvertebrates distribution patterns in the Segura River basin. This main gradient was correlated with the catchment longitudinal gradient, from freshwater, pristine, high altitude, humid headwaters in the NW to polluted lower reaches towards the more arid SE.

6. The most influential variables for community structure were variables that usually operate at a regional scale, affected by climatic and/or geological patterns (mineralization, altitude, rainfall) or land use activities (eg. Nitrate).

7. We found a highly significant relationship between both biological and ecological traits and the environmental features of the habitats, as measured by the main underlying environmental gradient.

8. The most influential variables were land use, riparian forest quality, geology and altitude. There was also a strong spatial influence. Electric conductivity, nitrate, water temperature and discharge also exerted major influences. Among the in-stream habitat variables, those related to substrate size and heterogeneity were the most influential, but to a lesser degree in comparison with the other variables.

9. Our results suggest that a variety of factors acting at different scales and hierarchically organized controls the functional organization of communities at our scale of study.

10. The functional diversity index has demonstrated to better perform in detecting impairment in stream macroinvertebrate communities in our region than taxa richness. Its lack of dependence on different natural factors that clearly affected other diversity measures as taxa richness or Shannon entropy, poses this newly developed diversity index as a good candidate metric in assessing human induced community changes in stream ecosystems.

11. The solid theoretical foundations over which functional diversity is based, offers the possibility of modifying the trait spectrum according to the objectives of a study, by choosing the particular features a priori involved in the specific change or process that we want to evaluate.