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# Bioassessment of organically polluted Spanish rivers, using a biotic index and multivariate methods

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*Abstract.* In this study we compared water-quality results obtained by a modified version of the Biological Monitoring Working Party Score System (BMWP', a multimetric method) with those from multivariate methods—TWINSPAN and Canonical Correspondence Analysis (CCA)—in a river basin in southern Spain affected by organic pollution. Main environmental variables correlated to macroinvertebrate distributions were identified by CCA. Biological quality of the water was assessed by the BMWP' biotic index, which classified the sites according to the tolerance of taxa to organic pollution. TWINSPAN was used to classify the sites according to benthic communities, and CCA was performed to establish the relationships among groups of sites, taxa, and abiotic variables. The results showed a clear separation of river sites according to their water composition and degree of pollution; nutrient content and water hardness were the main factors influencing the faunal distribution. Most of the sites in the study area were polluted, and consequently the most frequently encountered taxa were tolerant species. Moreover, the worsening of the water quality downstream was accompanied by a substitution of species, even within the same genus, from less to more pollution tolerant. The results obtained by the multivariate analyses used were highly satisfactory for correctly interpreting the assemblages and substitutions of macroinvertebrates, according to the main environmental variables measured in the basin. Moreover, the classification of sites according to macroinvertebrate requirements, provided by TWINSPAN analysis, was closely related to the classification according to tolerance to water pollution, provided by the biotic index. In addition, a significant part of the variance of each taxon as a pollution bioindicator, in the sites where they were found, was explained by the main canonical axis. Thus, the BMWP' biotic index, of easy applicability in river basins, proved to be an easy tool for water-quality assessment.

*Key words:* BMWP' biotic index, TWINSPAN, CCA, macroinvertebrates, indicator organism, rivers, environmental factors, hardness, organic pollution, Spain.

Benthic macroinvertebrates have been widely used as indicators of water quality in river management because, apart from many other advantages over using other organisms (e.g., Hellawell 1986, Metcalfe 1989, Jeffries and Mills 1990, Rosenberg and Resh 1993), they are affected not only by natural changes in the rivers but also by chemicals and physical factors induced by human activities (e.g., Hart and Fuller 1974, Wiederholm 1984, Hellawell 1986, Prat and Ward 1994). The Biological Monitoring Working Party (BMWP) Score System (Armitage et al. 1983), applied mainly in the UK, has been adapted to the Iberian Peninsula under the name of BMWP' (Alba-Tercedor and Sánchez-Ortega 1988). Because of the easy application of this index, high correlations with other European indices (Rico et al. 1992), its good relationships with variables indicative of pollution, and its reduced variability with respect to seasonality (Zamora-Muñoz et al. 1995), the BMWP' index was chosen for the purpose of this study. Furthermore, this index was adopted in September 1991 by the Spanish Society of Limnology for use throughout the Iberian Peninsula to assess water quality.

Multivariate techniques have been shown to be powerful methods in ecological studies on macroinvertebrates (Wright et al. 1984, Eyre et al. 1986, Leland et al. 1986, Bargos et al. 1990, Basaguren and Orive 1990, Kansanen et al. 1990, Palmer et al. 1991, Johnson et al. 1993, Gower et al. 1994). The main advantage is that these techniques reduce the multidimensional data with a minimum loss of information, making it possible to find directions of variability in the data.

In the present study, a Two-Way INdicator SPecies ANalysis (TWINSPAN; Hill 1979) was used to classify the sites according to their macroinvertebrate community, and a Canonical Correspondence Analysis (CCA; Ter Braak 1986, 1987), was used simultaneously to examine relationships between sites, species and environ-

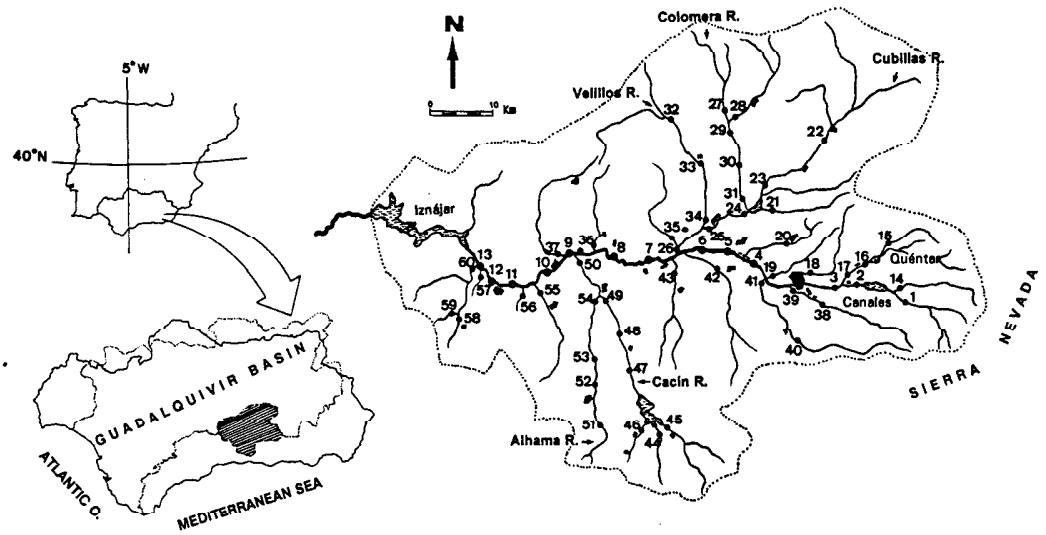


FIG. 1. Study area showing locations of the 60 sampling sites (black circles) and large villages and towns (irregular black marks) in the Genil River Basin. Only the main rivers in the basin and the reservoirs mentioned in the text are named. The Genil River is shown by a thick line (sites 1–13). The city of Granada lies between sites 18 and 39.

mental variables. TWINSPAN has been widely used in the field of limnology, especially for the classification of rivers based on their biotic assemblages (e.g., Wright et al. 1984, Ormerod and Edward 1987).

In an attempt to enhance the dialogue between biomonitoring and ecological research, the present study was conducted to compare the water-quality results obtained by the BMWP' index (a multimetric method) with those from multivariate methods (TWINSPAN and CCA), in river basin assumed to be affected by organic pollution. Furthermore, by CCA, we tried to identify the main environmental variables affecting macroinvertebrate distributions, as well as to validate the BMWP' by comparing the biological classification made by this index with the environmental ordination produced by CCA.

## Study Sites

The study was carried out in the upper Genil River Basin (4500 km²), from the spring source of the river (on the northwestern slope of the Sierra Nevada Mountains, southern Spain) to the Iznájar Reservoir. The basin water is regulated by 4 reservoirs (Fig. 1).

The headwaters of the rivers from the Sierra Nevada mountains flow over siliceous material (southeast of the basin), while the other rivers flow over calcareous material, except in the valleys of the middle reaches of the main rivers (Genil, Cubillas, Colomera, Velillos, Cacín, and Alhama), where there are detrital materials and, in some areas of the southwest of the basin, salt mines.

The upper Genil basin is affected by human activities, mainly urban waste water (most of the sewage during the study period was dumped without purification; Servicio Andaluz de Salud-Universidad de Granada 1991), agro-industrial pollution (mainly extraction waste from olive-oil factories in winter), and fish-hatchery and farm dumpings.

## Methods

### *Site selection*

We established a total of 60 sites on the main course of the Genil River, and the 26 tributaries. The sites were upstream and downstream of potential sources of pollution (towns or villages, factories, and river mouths; Fig. 1) and were chosen according to a map and visits to the area.

Except for a few headwaters sites without any nearby town (sites 1, 14, 15, 27, 38, 40, 44, and 51), most of the sites were expected to be affected by urban dumpings. The sites most affected by this kind of pollution were expected to be the sites downstream of the biggest towns and villages (4, 10, 12, 19, 22, 25, 26, 28, 39, 50, and 52). Sites 18 and 39, near the city of Granada, were also likely to be affected by industrial areas. Agricultural pollution was expected to affect sites in farming land (7–13, 32–36, 41–43, 48, 49, 55, 56, 58–60). A seasonal pollution (December to March) from olive pressing was expected to affect some sites, and a few sites were located in downstream reservoirs (sites 2, 16, 24, 29, and 47). Because urban sewage often includes agricultural and industrial pollutants, it can be difficult or impossible to distinguish the kinds of pollution affecting a site.

*Field and laboratory methods*

Field samples were taken during 4 seasons in 1988: 15–24 March, 14–25 June, 21–30 September and 9–20 December.

Macroinvertebrates were sampled qualitatively at each site, using 2 kinds of hand net: circular (25 cm frame diameter, 17 cm-deep bag, 900 μm mesh) and rectangular (20 × 15 cm frame, 18 cm-deep bag, 400 μm mesh). Two persons kicked and swept independently in all the different microhabitats (riffles, depositional zones, different types of vegetation). The contents of each netting were deposited periodically in white trays to avoid losing organisms by overflow from the nets. Because the range of site characteristics was wide, we would have lost information about taxa richness if we had standardized the sampling effort by time. Each sampling was considered finished when sweeps produced no new taxa at the family or genus-level identification (easily distinguished by a trained eye). Specimens were preserved in 70% ethanol for detailed identification in the laboratory. They were labelled and deposited in the collection of the Department of Animal Biology and Ecology (Section of Zoology) of the University of Granada.

For each site, the slope, altitude, and distance from the spring source (origin of the river) were derived from 1:50,000 Ordnance Survey maps. Water temperature was measured with a standard thermometer; and mean stream depth, width, and surface current velocity (a timed float) were recorded during each sampling to calculate the streamflow.

Water samples for each site in each season were collected in polyethylene bottles (2 bottles, each 1 L) and analyzed in the laboratory for the following variables: conductivity, nitrites, ammonium, phosphates, chlorides, calcium, magnesium, sodium, and potassium. Concentrations of nitrites, ammonium and phosphates were measured by spectrophotometry using a Bausch and Lomb spectrophotometer UV–VIS model Spectronic 2000. Chlorides were determined by titration with $AgNO_3$. Calcium, magnesium, sodium, and potassium concentrations were measured by atomic absorption spectrophotometry (Perkin Elmer mod. 2380), and conductivity with a Radiometer CDM–3.

*Biotic index and multivariate analyses*

The BMWP, a simple scoring method with all macroinvertebrate groups identified to the family level, requires only qualitative data. A site score is obtained by summing the individual scores of all families present (see table 1 in Armitage et al. 1983). Score values for individual families reflect their pollution tolerance based on current knowledge of distribution and abundance. Pollution-intolerant families have high scores and pollution-tolerant families have low scores (Armitage et al. 1983).

Biological quality of the water was assessed in this study by the biotic index BMWP', an adaptation of the British BMWP system for the Iberian Peninsula (Alba-Tercedor and Sánchez-Ortega 1988). Adaptations of the BMWP' included the addition of new families, changes in some scores, and correlations of BMWP' values with particular significances in degrees of pollution. Five classes of water quality were thereby established (Class I: BMWP' value > 101, lightly or unpolluted site—good situation; Class II: BMWP' value 61–100, slightly polluted—acceptable situation; Class III: BMWP' value 36–60, moderately polluted—doubtful situation; Class IV: BMWP' values 16–35, heavily polluted—critical situation; Class V: BMWP' value < 16, very heavily polluted—very critical situation). The original table from Alba-Tercedor and Sánchez-Ortega (1988) was modified to include 1 new indicator family (Thiaridae, score = 6).

Prior to the multimetric and multivariate

analyses, we excluded: 1) 14 sites for which there were no data for some samplings (because the sites were so polluted that they were devoid of macroinvertebrates or because in some seasons the course was dry or overflowing), and 2) taxa found in less than 4 samplings (less 2% of the total data set) in order to eliminate rare taxa.

To determine whether the data sets could be pooled in the absence of seasonal differences, Wilcoxon matched pair tests were performed for the environmental data and Cochran tests for the biological data (Siegel and Castellan 1988). The results showed that there were statistical differences between samplings, and thus a total of 184 sampling units (4 samplings from 46 sites) and 125 taxa (presence/absence data) formed the data set used for the subsequent multivariate analyses. To check for differences in taxonomic identification level with the comparison between the results of the canonical analyses and the values of BMWP' index (which requires only family identification), we summarized the presence/absence data at the family identification level (74 families), and repeated the analyses.

Sites were classified by TWINSPAN analysis (Hill 1979), which is a polythetic divisive method that operates in several steps where, at each step, the sites-by-species matrix is dichotomously divided. Hierarchical classification by TWINSPAN is generally effective and robust, requires less computation than agglomerative techniques, and is the best method when the data set is large, complex, and "noisy" (Gauch and Whittaker 1981). The analyses followed the default options (pseudospecies cut levels were: 0 2 5 10 20; minimum group size for a division was 5; maximum number of indicators per division was 7; maximum number of species in final table was 100; pseudospecies were weighted equally; all cut levels had equal indicator potential, and all species were considered potential indicators), and were run for 4 division levels. Later the groups were tested by Cochran tests to pool the groups that did not differ statistically.

Environmental variables were transformed to approximate normality. After a visual inspection of the frequency distribution of each variable, we tried different transformations for each variable to find the appropriate one to approximate a normal distribution. When frequency distributions skew to the right, the most common transformation is the conversion of the variable into logarithms. But, sometimes, this transformation does not work well, and it is necessary to try other power transformations, as $1/\sqrt{X}$, $\sqrt{X}$, $1/X$, ... for samples skewed to the right, and the transformations $X^2$, $X^3$, ... for samples skewed to the left (Sokal and Rohlf 1995). The criteria applied to choose the appropriate transformation were: 1) Kolmogorov-Smirnov test for normality applied to the transformed distribution was non-significant ($p > 0.05$), and 2) skewness and kurtosis coefficients of this distribution of frequency were the closest to their critical values for $n = 184$ and $p < 0.01$ ($\pm 0.18$ for skewness and $\pm 0.36$ for kurtosis). The untransformed temperature was normal; the rest of the variables needed an optimal transformation: calcium: $1/\sqrt[10]{\log(x + 20)}$; magnesium: $1/\sqrt{\log(x + 20)}$; chlorides: $1/\sqrt[6]{(x + 1)}$; sodium: $\log(x + 1)$; potassium: $1/\sqrt[3]{(x + 2)}$; altitude: $1/x$; flow and conductivity: $\log(x)$; nitrites: $\sqrt{\log(x + 1)}$; ammonium: $1/\sqrt{\log(x + 2.5)}$; phosphates: $1/\sqrt{\log(x + 2)}$; distance from spring source: $1/\sqrt[6]{(x + 5)}$; slope: $1/\sqrt[6]{(x)}$.

The species-environment relationships of the data were analyzed by a Canonical Correspondence Analysis (CCA), using the CANOCO program version 3.12 (Ter Braak 1991). CCA is a powerful tool for simplifying complex data sets and, being a direct gradient analysis, allows integrated analysis of both taxa and environmental data (Ter Braak 1986, 1987). The technique identifies an environmental basis for community ordination by detecting the patterns of variation in community composition that can be explained best by the environmental variables. Two CCAs were performed, one with biological data identified to maximum level (most taxa to species or genus level) and another to family identification level only. The option symmetric scaling of samples and species scores was selected for better diagrammatic results. The rest of the analysis followed the default options (no forward selection of the environmental variables; species and sample diagnostics by chi-square fit, residual distances and tolerances; no sample or environmental variable omitted; no product of environmental variables; no transformation of the species data, no weight for species and samples, and no downweighting of rare species, because we had already eliminated most of them in a previous step).

Weighted intraset correlations of environmen-

tal variables with axes are the correlation coefficients between the environmental variables and the ordination axes. Weighted intraset coefficient were used to infer the relative importance of each environmental variable for predicting the community composition. Although some of the environmental variables used in the analyses were correlated with others (e.g., variables indicative of hardness of the waters), there was no strong multicollinearity among the variables (variables with a Variance Inflation Factor close to 20); therefore, none were eliminated or made passive. Species-environment correlation coefficients provide a measure of how well the extracted variation in community composition can be explained by the environmental variables. Monte Carlo permutation tests were used to assess the significance of the canonical axes. Names of the sites in CCA diagrams were coded using respective TWINSPAN groups.

To test if the results of the BMWP' index were related with those obtained by the CCA with the environmental data, we performed another CCA using BMWP' index as the only explanatory variable and compared the 1st canonical axes of each analysis. A Correspondence Analysis (CA), with only taxa data, was also performed to see what was the maximum amount of variation that can be explained by a single axis. Comparing the results of the CA and the CCAs we could assess if the measured environmental variables and the BMWP' index accounted for the main directions of variations in the species data.

Moreover, in the attempt to validate the classification made by the BMWP' index with the environmental ordination produced by CCA, we performed a multiple regression between the established tolerance value of each taxon as a pollution bioindicator (from the score table of BMWP'), in the sites where we found them (dependent variable), and the taxa scores on the different axes obtained by the canonical ordination (independent variables).

## Results

A total of 74 macroinvertebrate families and 125 taxa occurring in ≥4 samplings were included in 2 sets of TWINSPAN and CCA analyses. Most taxa were identified to species, but some were identified to subfamily, tribe, or genus because we lacked Iberian keys for some groups (e.g., some Diptera) and presence of only immature forms. In 9 cases the identifications were only to the family level (Lumbriculidae, Tubificidae, Naididae, Erpobdellidae, Cypridae, Psychodidae, Simuliidae, Stratiomyidae and Helodidae). Hydracarina and 3 taxa corresponding to unidentified Coleopteran larvae (Dytiscidae, Gyrinidae and *Limnius* larvae), were retained in the analyses. Most species were insects (72 of the 91 species identified in total); and although insects are better known taxonomically than oligochaetes, for example, the high number of species is a reasonable measure of insect dominance given that Diptera were not identified to species level, either. Among insects, and among the taxa that were identified to species level, the richest orders were Ephemeroptera (23 species, 9 belonging to the family Baetidae) followed by Coleopteran (18 species) and Trichoptera (17 species, of which 7 were Hydropsychidae).

Because the TWINSPAN analysis is a divisive technique, the division of the samples into progressively smaller groups may be terminated at any level considered appropriate. After 4 division levels, the analysis of the data set with taxa identified to the maximum level (125 taxa) produced 16 groups of samplings, however, only 12 groups were considered because 4 divisions were not statistically different, according to Cochran tests, and were pooled. Table 1 gives the percentage of frequency of occurrence, based on presence/absence data, of "indicator species" (see Hill 1979) in each TWINSPAN group, as well as the average taxa richness and BMWP' index for the groups. Table 2 presents the sampling sites belonging to each of the 12 TWINSPAN groups. In the 1st division, sites were selected on the basis of the presence or absence of *Perla marginata* and *Hydropsyche instabilis*. These 2 species occurred almost exclusively in sites of groups 7, 12, 26, and 27 (*P. marginata* was also found at sites of group 19, but in only 13% of these). In those groups (7, 12, 26, and 27), *Baetis rhodani*, *Ecdyonurus* sp., *Elmis maugetii* and *Rhyacophila nevada* were also major taxa (Table 1). The remaining sites were grouped according to other indicator species, which in the 2nd division were: *Elmis maugetii*, *Hydropsyche infernalis*, *Echinogammarus simoni*, *Baetis pavidus*, and *Caenis luctuosa*. On one hand, the following divisions resulted in groups 16–19 that, except for group 16 (formed by 1 sampling of 1 site, with 4 taxa only), had in common

(at a high frequency) the species *E. maugetii, H. infernalis,* and *Chironomus* gr. *thummi.* On the other hand, in groups 20–23, *B. pavidus* together with *Caenis luctuosa* were dominant. Groups 7, 26, and 12 had the highest values of mean taxa richness and biotic index, followed by groups 22, 27, and 17, which, although having a lower mean richness, registered a high mean value of the BMWP' index, indicative of good or acceptable water quality. Groups 19 and 21, despite having similar mean taxa richness, which was greater than that of groups 17 and 27, had a mean biotic index that was lower and representative of doubtful water quality. The TWINSPAN groups 17 and 27 were formed by sites holding taxa with higher scores in the BMWP' index (stoneflies, mayflies except Baetidae, Ephemerellidae, and Oligoneuriidae, and several families of case-bearing caddisflies) than the taxa found in the sites of groups 19, 21, and even 22. Groups 16, 18, 20, and 23, the most polluted sites, had the lowest values of richness and of BMWP' index, given that most taxa found in those sites have the lowest scores in the BMWP' index (oligochaetes, molluscs, many Diptera and beetles, Baetidae, and filter-feeding caddisflies).

It is noteworthy that a single site often appeared in more than 1 TWINSPAN group, generally those sites belonging to more polluted groups. Extreme examples were sites 18, 24, and 42, for which each season was classified in a different group (see Table 2).

To determine whether this classification of the sites by TWINSPAN analysis was statistically related to that obtained by the tolerance of the taxa to water pollution, we first ordered the 12 TWINSPAN groups according to their average BMWP' value and subsequently compared them with the BMWP' values of each site classified according to the 5 water-quality classes established for this index (see Methods; Table 3). TWINSPAN groups were ordered from having non-polluted or slightly polluted sites to very polluted sites (Table 3), and the classification of the sites based on ecological requirements of macroinvertebrate taxa by TWINSPAN analysis showed a highly positive correlation with the classification based on tolerance to water pollution (Pearson Chi-square = 232.77, df = 44, $p < 0.0001$; Spearman Rank correlation: $r_s = 0.82$, $p < 0.0001$).

TWINSPAN analysis for the data set summarized at the family level provided 10 statistically different groups. The classification of the sites gave different results because of the different identification level reached. However, in essence, those results were similar to the former: 5 groups of sites with good or acceptable water quality, 3 groups with average values of the biotic index indicative of doubtful water quality, and 2 groups with heavily polluted sites. Therefore, to avoid repetition, we shall not give details of TWINSPAN results with family identification. Like the species TWINSPAN analysis, the classification of the sites by TWINSPAN at the family level showed a highly positive correlation with the classification based on taxa tolerance to water pollution (Pearson Chi-square = 212.31, df = 36, $p < 0.0001$; Spearman Rank correlation: $r_s = 0.83$, $p < 0.0001$).

As in the classification analyses, 2 ordination (CCA) analyses were performed with our data set, one with taxa identified to genus or species level, and another with taxa identified only to family level. In both CCAs the 14 environmental variables were incorporated, and the results were good and highly similar in both cases (Table 4). Although the 4 resulting canonical axes were significant by Monte Carlo permutation tests, Axis 1 proved most explanatory (Table 4).

Because of the similar results between the 2 data treatments, only the CCA diagrams for maximum taxa identification were plotted. The results of CCA are displayed in ordination diagrams. In Fig. 2, sites (coded by their respective TWINSPAN group) are represented by squares, and environmental variables represented by arrows. In this biplot, the length of each arrow indicates the importance of the corresponding environmental variable for community composition, and its direction the maximum change of that variable (see Ter Braak 1986 for details in the ordination diagram interpretation). In Fig. 3, taxa are represented by squares.

TWINSPAN groups are spread along Axis 1 (Fig. 2), which clearly corresponds to a gradient of water mineralization and pollution, reflecting natural changes in the physico-chemical environment and human disturbance. Axis 1 proved to have a strong positive correlation with conductivity and other variables indicative of hardness of the waters (calcium, magnesium, chlorides, potassium, sodium), and pollution indicator variables (nitrites, ammonium, and phosphates), as well as a negative correlation with

TABLE 1. Percentage of frequency of occurrence (%), based on presence/absence data, of "indicator species" in the entire data set (184 samplings) and in each TWINSPAN group (G7–G27). Mean (and SD) taxon richness, mean (and SD) value of the BMWP' biotic index, and the number of samplings per group are also given.

| | | TWINSPAN groups | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | G7 | G12 | G16 | G17 | G18 | G19 | G20 | G21 | G22 | G23 | G26 | G27 |
| Gastropoda | | | | | | | | | | | | | |
| *Ancylus fluviatilis* | 60.9 | — | 76.2 | — | 40.0 | — | 25.0 | 31.8 | 17.4 | 38.1 | 8.3 | 75.0 | 16.7 |
| *Physella acuta* | 52.2 | — | 4.8 | — | 20.0 | 20.0 | 50.0 | 61.4 | 73.9 | 28.6 | 8.3 | — | — |
| *Melanopsis dufouri* | 8.7 | — | — | — | 80.0 | — | 12.5 | 2.3 | — | — | 8.3 | — | — |
| Oligochaeta | | | | | | | | | | | | | |
| *Eiseniella tetrahedra* | 73.9 | 21.4 | 57.1 | 100 | 100 | 20.0 | 62.5 | 27.3 | 34.8 | 14.3 | — | 83.3 | 66.7 |
| Naididae | 89.1 | 35.7 | 66.7 | — | — | 20.0 | 75.0 | 47.7 | 47.8 | 33.3 | 45.8 | 58.3 | 50.0 |
| Achaeta | | | | | | | | | | | | | |
| Erpobdellidae | 69.6 | — | 47.6 | — | 60.0 | — | 87.5 | 52.3 | 65.2 | 28.6 | 4.2 | 41.7 | 33.3 |
| Crustacea | | | | | | | | | | | | | |
| *Echinogammarus simoni* | 13.0 | — | 9.5 | — | 80.0 | — | 75.0 | 2.3 | 4.3 | — | — | — | — |
| *Asellus aquaticus* | 23.9 | — | — | — | — | 20.0 | — | 63.6 | 13.0 | 9.5 | — | — | — |
| Cypridae | 69.6 | 21.4 | 57.1 | — | — | 40.0 | 25.0 | 27.3 | 87.0 | 42.9 | 29.2 | — | — |
| Insecta | | | | | | | | | | | | | |
| Ephemeroptera | | | | | | | | | | | | | |
| *Baetis fuscatus* | 30.4 | 71.4 | 9.5 | — | — | — | — | 4.5 | — | 33.3 | 37.5 | — | — |
| *B. pavidus* | 80.4 | 35.7 | 38.1 | — | 20.0 | 20.0 | 25.0 | 77.3 | 78.3 | 100 | 91.7 | — | — |
| *B. rhodani* | 80.4 | 92.9 | 100 | — | 100 | — | 87.5 | 22.7 | 47.8 | 85.7 | 33.3 | 100 | 100 |
| *Cloeon cognatum* | 30.4 | 7.1 | — | — | — | — | — | 2.3 | 56.5 | 9.5 | 25.0 | — | — |
| *Caenis luctuosa* | 58.7 | 42.9 | 19.0 | — | — | — | 12.5 | 29.5 | 65.2 | 66.7 | 66.7 | — | — |
| *Ecdyonurus* sp. | 45.7 | 71.4 | 61.9 | — | 80.0 | — | 12.5 | — | — | 28.6 | 16.7 | 75.0 | 33.3 |
| *Epeorus sylvicola/torrentium* | 13.0 | 14.3 | — | — | — | — | — | — | — | — | — | 66.7 | 66.7 |
| *Rhithrogena* gr. *semicolorata* | 8.7 | 7.1 | — | — | — | — | — | — | — | — | — | 8.3 | 100 |
| Odonata | | | | | | | | | | | | | |
| *Ischnura* sp. | 26.1 | 7.1 | 9.5 | 100 | 60.0 | — | 12.5 | 2.3 | 34.8 | — | — | — | — |
| *Onychogomphus uncatus* | 10.9 | 64.3 | — | — | — | — | — | — | — | 4.8 | — | — | — |
| Plecoptera | | | | | | | | | | | | | |
| *Perla marginata* | 30.4 | 100 | 47.6 | — | — | — | 12.5 | — | — | — | — | 100 | 100 |

TABLE 1. Continued.

| | | TWINSPAN groups | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | G7 | G12 | G16 | G17 | G18 | G19 | G20 | G21 | G22 | G23 | G26 | G27 |
| Heteroptera | | | | | | | | | | | | | |
| *Micronecta* spp. | 45.7 | 35.7 | 14.3 | — | — | — | — | 6.8 | 17.4 | 57.1 | 70.8 | — | — |
| Coleoptera | | | | | | | | | | | | | |
| *Potamonectes clarcki* | 41.3 | 28.6 | 33.3 | — | — | 40.0 | — | 6.8 | 56.5 | 28.6 | 12.5 | 16.7 | — |
| *Elmis maugetii* | 37.0 | 14.3 | 61.9 | — | 100 | 60.0 | 75.0 | — | — | 4.8 | 4.2 | 58.3 | 83.3 |
| Coleoptera | | | | | | | | | | | | | |
| *Haliplus lineatocollis* | 50.0 | 14.3 | 38.1 | — | — | — | — | 15.9 | 69.6 | 23.8 | 16.7 | — | — |
| Trichoptera | | | | | | | | | | | | | |
| *Agapetus incertulus* | 13.0 | 14.3 | 4.8 | — | 80.0 | — | — | — | 4.3 | 4.8 | — | — | — |
| *Hydropsyche infernalis* | 32.6 | — | 14.3 | — | 100 | 80.0 | 75.0 | 4.5 | 17.4 | 9.5 | 4.2 | 8.3 | — |
| *H. instabilis* | 23.9 | 42.9 | 81.0 | — | — | — | — | — | — | — | — | 75.0 | 100 |
| *H. pellucidulla* | 45.7 | 71.4 | 28.6 | — | — | — | — | 6.8 | 4.3 | 71.4 | 37.5 | 75.0 | — |
| *H.* cf. *punica* | 41.3 | 50.0 | 33.3 | — | — | — | 12.5 | 4.5 | 34.8 | 71.4 | 4.2 | — | — |
| *Athripsodes* sp. | 10.9 | 7.1 | — | — | 80.0 | — | — | — | — | — | — | 50.0 | 33.3 |
| *Rhyacophila nevada* | 28.3 | — | 76.2 | — | — | — | — | — | — | 14.3 | — | 83.3 | 83.3 |
| Diptera | | | | | | | | | | | | | |
| *Chironomus* gr. *tiummi* | 58.7 | 7.1 | 9.5 | 100 | — | 40.0 | 50.0 | 56.8 | 39.1 | 4.8 | 12.5 | — | — |
| Tanypodinae | 63.0 | 57.1 | 57.1 | — | 20.0 | — | 25.0 | — | 52.2 | 52.4 | 25.0 | 33.3 | — |
| *Hexatoma* sp. | 23.9 | 71.4 | — | — | — | — | 12.5 | 2.3 | 4.3 | — | — | 91.7 | 33.3 |
| *Tipula* sp. | 60.9 | 78.6 | 57.1 | — | — | 40.0 | 12.5 | 13.6 | 21.7 | 61.9 | 4.2 | 8.3 | 50.0 |
| Psychodidae | 30.4 | 42.9 | 28.6 | — | 20.0 | — | 12.5 | — | 17.4 | 19.0 | — | 8.3 | 100 |
| Taxon richness | Mean | 31.4 | 27.4 | 4.0 | 19.8 | 8.8 | 20.3 | 12.5 | 20.5 | 22.0 | 13.4 | 25.5 | 20.0 |
| | SD | (5.8) | (5.4) | (—) | (2.2) | (5.0) | (5.9) | (4.7) | (3.8) | (5.7) | (4.2) | (4.2) | (5.0) |
| BMWP′ index | Mean | 126.9 | 96.8 | 16.0 | 86.2 | 28.8 | 60.3 | 31.2 | 54.7 | 63.5 | 34.1 | 97.8 | 85.5 |
| | SD | (23.4) | (22.6) | (—) | (13.1) | (20.7) | (21.3) | (16.0) | (14.4) | (18.2) | (10.4) | (15.0) | (22.1) |
| No. of samplings | | 14 | 21 | 1 | 5 | 5 | 8 | 44 | 23 | 21 | 24 | 12 | 6 |

TABLE 2. The resulting 12 groups of samplings classified by TWINSPAN analysis (G7–G27) from a data set of 184 samplings (46 sites × 4 samplings) and 125 taxa. The number of the site is followed by the number of the sampling in parentheses (1 to 4). See Fig. 1 for the position of each site in the study area.

| G7 | G12 | G16 | G17 | G18 | G19 | G20 | G21 | G22 | G23 | G26 | G27 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 (1–4) | 2 (1–4) | 42 (4) | 18 (4) | 42 (2) | 18 (2) | 11 (2, 3, 4) | 18 (3) | 29 (1, 2, 4) | 11 (1) | 1 (1–4) | 38 (1, 2, 4) |
| 27 (1–4) | 3 (1–4) | | 56 (1–4) | 43 (1, 2, 3) | 55 (1) | 12 (1–4) | 22 (1–4) | 30 (1–4) | 9 (3) | 14 (1–4) | 40 (1, 2, 4) |
| 44 (1–4) | 16 (1–4) | | | 52 (1) | 58 (1–4) | 6 (1–4) | 25 (3) | 31 (4) | 24 (1, 2) | 38 (3) | |
| 51 (3, 4) | 17 (1–4) | | | | 59 (2, 3) | 7 (1–4) | 28 (1, 3, 4) | 32 (2) | 29 (3) | 40 (3) | |
| | 18 (1) | | | | | 8 (1–4) | 31 (3) | 33 (1, 2) | 34 (1) | 51 (1, 2) | |
| | 47 (4) | | | | | 9 (1, 2, 4) | 32 (1, 3, 4) | 34 (4) | 45 (1, 2) | | |
| | 59 (1, 4) | | | | | 24 (3, 4) | 33 (3, 4) | 47 (1, 2, 3) | 49 (2, 3, 4) | | |
| | 60 (1) | | | | | 25 (1, 2, 4) | 34 (3) | 49 (1) | 50 (1–4) | | |
| | | | | | | 26 (1–4) | 37 (2, 3, 4) | 52 (2) | 53 (1–4) | | |
| | | | | | | 28 (2) | 42 (3) | 54 (4) | 54 (1, 2, 3) | | |
| | | | | | | 34 (2) | 43 (4) | 60 (2, 3, 4) | | | |
| | | | | | | 37 (1) | 45 (3) | | | | |
| | | | | | | 42 (1) | 55 (3) | | | | |
| | | | | | | 45 (4) | | | | | |
| | | | | | | 52 (3, 4) | | | | | |
| | | | | | | 55 (2, 4) | | | | | |
| | | | | | | 57 (1–4) | | | | | |

TABLE 3. Number of sites from each of the 12 TWINSPAN groups (from the analysis with 125 taxa) belonging to each of the 5 water quality classes established for the BMWP' index. The TWINSPAN groups were first ordered by their respective BMWP' average values.

| | Water quality class | | | | |
|---|---|---|---|---|---|
| TWINSPAN groups | 1 | 2 | 3 | 4 | 5 |
| 7 | 13 | 1 | | | |
| 26 | 6 | 6 | | | |
| 12 | 11 | 8 | 2 | | |
| 17 | 1 | 4 | | | |
| 27 | 2 | 4 | | | |
| 22 | | 12 | 7 | 2 | |
| 19 | | 4 | 3 | 1 | |
| 21 | | 9 | 13 | 1 | |
| 23 | | | 10 | 14 | |
| 20 | | 1 | 17 | 18 | 8 |
| 18 | | | 3 | | 2 |
| 16 | | | | 1 | |

the slope and altitude of the sites (Table 5). Groups of sites in the lower left quadrant of the diagram (G26 and G27) correspond to headwater sites of the Genil and other rivers of the Sierra Nevada Mountains (siliceous rock), with strong slope and low conductivity. Groups 7 and 12, left upper and lower quadrant, are formed by sites from the Genil River downstream from the Canales Reservoir, sites of Aguas Blancas River upstream and downstream from Quéntar Resevoir, and upper reaches of rivers flowing over calcareous rock. Groups on the right of the diagram are from the middle and lower reaches of the Genil River as well as middle stretches and the mouths of polluted tributaries (see Table 6 for mean values of environmental variables of the groups). Sites with higher water mineralization belong to groups 16 and 18, and the most polluted to groups 18, 19, and 20 (Table 6). Sample ordination scores on Axis 1 had a negative correlation with taxa richness ($r = -0.62$, $p < 0.0001$). In the case of family identification level, a similar result was given by sample ordination scores on Axis 1 correlation with taxa richness ($r = -0.60$, $p < 0.0001$).

Axis 2 was negatively related to water flow (Table 5), so that, among the sites with hard waters and higher pollution (those to the right side of the Fig. 2), most of the sites of group 20 were separated from the rest of the groups, because that group included the lower reaches of Genil River (Fig. 1 and Table 2), with sites farther

TABLE 4. Results of canonical correspondence analysis (CCA) using all environmental variables, with taxa identified to maximum level (125 taxa) and with family identification level (74 families): eigenvalues of 4 axes, species-environment correlations with the axes, total variance in species data (total inertia), cumulative percentage variance accounted for by the axis of the species-environment relation, and results of Monte Carlo tests for each axis.

| | Axes | | | | Total |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | inertia |
| 125 taxa | | | | | |
| Eigenvalues | 0.352 | 0.126 | 0.111 | 0.082 | 4.903 |
| Species-environment correlations | 0.927 | 0.819 | 0.782 | 0.741 | |
| Cumulative percentage variance | | | | | |
| of species data | 7.2 | 9.7 | 12.0 | 13.7 | |
| of species-environment relation | 34.6 | 47.0 | 57.9 | 66.0 | |
| Sum of all canonical eigenvalues | 1.016 | | | | |
| Monte Carlo permutation test $F$ ($p < 0.01$) | 13.06 | 4.83 | 4.66 | 3.65 | |
| 74 families | | | | | |
| Eigenvalues | 0.264 | 0.096 | 0.076 | 0.060 | 3.552 |
| Species-environment correlations | 0.908 | 0.761 | 0.745 | 0.648 | |
| Cumulative percentage variance | | | | | |
| of species data | 7.4 | 10.1 | 12.3 | 13.9 | |
| of species-environment relation | 34.1 | 49.2 | 59.6 | 67.8 | |
| Sum of all canonical eigenvalues | 0.730 | | | | |
| Monte Carlo permutation test $F$ ($p < 0.01$) | 13.55 | 5.14 | 4.38 | 4.15 | |

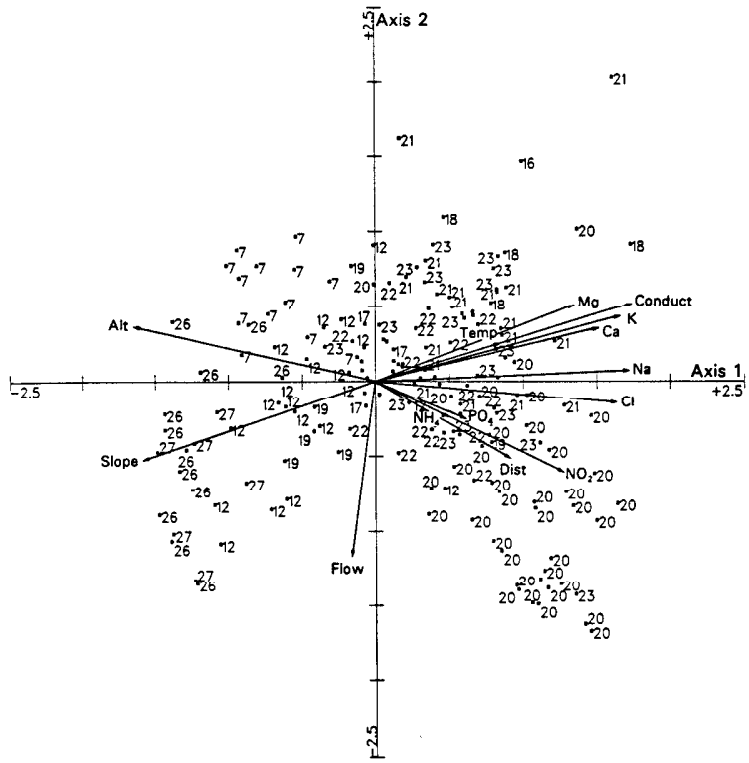


FIG. 2. CCA ordination diagram of sites classified according to their TWINSPAN groups and environmental variables (arrows). The environmental variables are: Ca (calcium concentration), Mg (magnesium concentration), Cl (chloride concentration), Na (sodium concentration), K (potassium concentration), Alt (altitude), Temp (temperature), Flow, Conduct (conductivity), $NO_2$ (nitrite concentration), $NH_4$ (ammonium concentration), $PO_4$ (orthophosphate concentration), Dist (distance of site from the spring source), Slope (slope of the site).

TABLE 5. Weighted intraset correlations of environmental variables with the axes of canonical correspondence analysis (CCA) for 125 taxa. Variable names preceded by an asterisk refer to variables inversely transformed.

| Variable | Axis 1 | Axis 2 | Axis 3 | Axis 4 |
|---|---|---|---|---|
| *Calcium (mg/L) | −0.7372 | −0.2277 | −0.0255 | −0.1452 |
| *Magnesium (mg/L) | −0.6358 | −0.3099 | 0.0338 | −0.3770 |
| *Chlorides (mg/L) | −0.7927 | 0.0878 | −0.1054 | 0.0992 |
| Sodium (mg/L) | 0.8299 | 0.0427 | 0.0509 | −0.2091 |
| *Potassium (mg/L) | −0.8068 | −0.2667 | 0.2645 | −0.0070 |
| *Altitude (m) | 0.7213 | −0.2128 | 0.4330 | 0.0867 |
| Temperature (°C) | 0.4083 | 0.1594 | 0.0064 | −0.2008 |
| Flow ($m^3/s$) | −0.0767 | −0.7221 | 0.1872 | 0.3197 |
| Conductivity (μS/m) | 0.8410 | 0.3264 | 0.0475 | 0.0545 |
| NO2 (mg/L) | 0.6094 | −0.3654 | −0.1122 | −0.3718 |
| *NH4 (mg/L) | −0.2447 | 0.1482 | −0.1483 | −0.0114 |
| *PO4 (mg/L) | −0.2992 | 0.1603 | 0.0002 | 0.5020 |
| *Distance from source (km) | −0.4526 | 0.3174 | 0.7334 | −0.2853 |
| *Slope | 0.7696 | 0.3258 | 0.1418 | 0.1163 |

TABLE 6. Mean (± 1 SD) of environmental variables in each of the 12 TWINSPAN groups (G7–G27) for 125 taxa.

| TWINSPAN groups | Calcium (mg/L) | Magnesium (mg/L) | Chlorides (mg/L) | Sodium (mg/L) | Potassium (mg/L) | Altitude (m) | Temperature (°C) |
|---|---|---|---|---|---|---|---|
| G7 | 61.21 (26.86) | 36.29 (15.00) | 12.93 (8.93) | 3.93 (3.27) | 1.43 (0.85) | 900.00 (109.83) | 12.57 (4.35) |
| G12 | 53.57 (22.36) | 21.52 (12.53) | 45.43 (100.31) | 30.62 (72.52) | 1.57 (1.25) | 751.43 (123.22) | 12.81 (3.67) |
| G16 | 581.00 (0.00) | 327.00 (0.00) | 3003.00 (0.00) | 563.00 (0.00) | 19.00 (0.00) | 545.00 (0.00) | 3.00 (0.00) |
| G17 | 65.80 (7.66) | 24.60 (6.77) | 27.00 (14.00) | 8.60 (2.88) | 2.20 (2.68) | 544.00 (98.39) | 14.10 (4.02) |
| G18 | 496.60 (248.86) | 165.80 (86.07) | 542.60 (890.23) | 310.00 (544.14) | 13.80 (13.35) | 589.00 (106.79) | 16.30 (2.73) |
| G19 | 67.25 (18.69) | 21.63 (13.18) | 124.13 (200.02) | 23.63 (16.69) | 1.63 (2.00) | 525.00 (79.10) | 15.25 (1.56) |
| G20 | 141.98 (86.35) | 57.07 (36.38) | 162.45 (385.36) | 97.34 (210.73) | 4.59 (3.48) | 540.80 (90.62) | 15.39 (3.95) |
| G21 | 158.04 (123.51) | 67.61 (75.29) | 204.70 (609.53) | 144.70 (470.87) | 6.26 (5.51) | 681.09 (135.61) | 14.17 (5.64) |
| G22 | 113.52 (63.21) | 34.95 (23.33) | 92.81 (52.14) | 42.14 (27.89) | 3.24 (1.48) | 653.33 (112.13) | 14.21 (5.10) |
| G23 | 126.92 (55.97) | 49.92 (28.68) | 75.21 (78.72) | 34.13 (44.97) | 4.00 (1.35) | 625.42 (107.82) | 15.23 (3.85) |
| G26 | 28.17 (16.39) | 10.08 (7.74) | 10.67 (8.79) | 2.17 (1.19) | 0.17 (0.39) | 1075.00 (110.25) | 10.71 (4.07) |
| G27 | 31.83 (13.59) | 9.83 (4.96) | 15.50 (12.16) | 3.17 (1.33) | 0.33 (0.52) | 1030.00 (54.77) | 7.75 (3.37) |

TABLE 6. Extended.

| TWINSPAN groups | Flow ($m^3/s$) | Conductivity ($\mu S/m$) | $NO_2$ (mg/L) | $NH_4$ (mg/L) | $PO_4$ (mg/L) | Distance (Km) | Slope |
|---|---|---|---|---|---|---|---|
| G7 | 375.71 (272.64) | 471.29 (49.36) | 0.03 (0.05) | 0.71 (0.86) | 0.19 (0.26) | 11.39 (0.68) | 0.04 (0.01) |
| G12 | 928.67 (930.90) | 570.86 (505.43) | 0.28 (0.28) | 0.99 (0.88) | 0.33 (0.29) | 19.01 (5.12) | 0.05 (0.03) |
| G16 | 55.00 (0.00) | 11,740.00 (0.00) | 0.07 (0.00) | 0.00 (0.00) | 0.80 (0.00) | 12.10 (0.00) | 0.01 (0.00) |
| G17 | 558.60 (198.09) | 493.80 (19.04) | 0.16 (0.12) | 1.52 (1.82) | 0.35 (0.32) | 5.08 (7.11) | 0.02 (0.01) |
| G18 | 67.20 (99.51) | 4062.40 (3032.60) | 1.13 (0.94) | 26.38 (21.60) | 1.73 (1.39) | 15.44 (2.52) | 0.02 (0.01) |
| G19 | 787.00 (724.30) | 805.50 (649.05) | 0.52 (0.81) | 2.30 (2.19) | 1.18 (1.15) | 8.23 (6.88) | 0.04 (0.02) |
| G20 | 3231.20 (3419.90) | 1382.60 (1470.30) | 1.55 (1.52) | 3.92 (7.35) | 0.92 (1.51) | 52.89 (30.49) | 0.02 (0.01) |
| G21 | 151.65 (213.97) | 1719.90 (2578.50) | 0.75 (0.83) | 1.16 (1.46) | 0.83 (1.27) | 21.89 (8.12) | 0.02 (0.01) |
| G22 | 654.38 (791.91) | 1014.10 (377.85) | 0.69 (0.85) | 2.92 (6.85) | 0.70 (0.81) | 21.97 (8.05) | 0.03 (0.01) |
| G23 | 759.58 (2035.20) | 1119.60 (480.91) | 0.37 (0.59) | 1.25 (1.31) | 0.38 (0.45) | 37.85 (18.71) | 0.02 (0.01) |
| G26 | 617.33 (400.70) | 189.58 (116.17) | 0.03 (0.06) | 0.50 (0.58) | 0.17 (0.25) | 14.88 (2.78) | 0.10 (0.03) |
| G27 | 687.67 (503.58) | 190.50 (70.17) | 0.10 (0.08) | 1.28 (1.75) | 0.44 (0.40) | 16.60 (2.08) | 0.11 (0.00) |

TABLE 7. Results of correspondence analysis (CA) and canonical correspondence analysis (CCA) using BMWP' index as environmental variable, with taxa identified to maximum level (125 taxa) and with family identification level (74 families).

| | CA | CCA |
|---|---|---|
| 125 taxa | | |
| Eigenvalue Axis 1 | 0.416 | 0.282 |
| Sum of all canonical eigenvalues | 4.903 | 0.282 |
| 74 families | | |
| Eigenvalue Axis 1 | 0.330 | 0.230 |
| Sum of all canonical eigenvalues | 3.552 | 0.230 |

from the spring source (Table 6). Moreover, among the soft water and non-polluted or slightly-polluted sites (those to the left of the Fig. 2), groups 26 and 27 (and a few sites of group 12), with steep slope and high-water-volume sites from Sierra Nevada mountain streams, were situated lower in the diagram (Fig. 2). Sample ordination scores on Axis 2 had a positive correlation with taxa richness ($r = 0.44$, $p < 0.0001$). For family identification level, sample scores on Axis 2 correlation with taxa richness ($r = 0.32$, $p < 0.0001$) was similar.

The most polluted groups of sites were groups 18 and 20 that correspond to sites of the Genil River between the city of Granada and the Iznájar Reservoir and tributaries affected by the dumping of urban, industrial, and agricultural wastes (Fig. 1 and Table 2). Moreover, groups 18 and 16 were mainly formed for a single river (Table 2) that flows over saline material, giving rise to the high average conductivities (Table 6).

Even when the 4 samplings from 1 site were classified in different groups by TWINSPAN analysis (see before and Table 2), the environmental ordination of samplings by CCA showed that those sites tended to lie together in a particular part of the diagram. For example, points of the groups 16, 18, 20, and 21 situated in the upper right quadrant of the Fig. 2, correspond to the 4 samplings of site 42.

After comparing the sample scores of the 1st canonical axis of CCA (the most explanatory axis) with those of Axis 1 of a CA (Table 7), we found that a highly significant part of the variation in the species data was accounted for by the environmental variables measured, both for species identification level (Linear Regression analysis; $R^2 = 0.80$, $F(1, 182) = 721.59$, $p < 0.0001$) and for family level analysis (Linear Regression analysis; $R^2 = 0.73$, $F(1, 182) = 479.62$, $p < 0.0001$).

Running a CCA for species identification level using BMWP' index as the only explanatory variable, and comparing the sample scores for the 1st axis (Table 7) with those of the CCA using all environmental variables measured and the CA, we found that Axis 1 of CCA with BMWP' as variable was negatively correlated with Axis 1 of CCA with all environmental variables ($r = -0.73$, $p < 0.0001$) and positively correlated with Axis 1 of CA ($r = 0.77$, $p < 0.0001$). The same trend and similar results were found when using the family identification level: Axis 1 of CCA with BMWP' as variable (Table 7) was negatively correlated with Axis 1 of CCA with all environmental variables ($r = -0.74$, $p < 0.0001$) and positively correlated with Axis 1 of CA ($r = 0.78$, $p < 0.0001$). These results showed that BMWP' index responds to a gradient of pollution in the sites. The sites with higher BMWP' values were inversely related to variables indicative of pollution.

The drop in the eigenvalues of CA and the 2 CCAs performed from the maximum identification level to the family level (Table 4, 7), indicated that a great deal of variation in the data set is lost when the data set is reduced from 125 taxa to 74 families.

The closest groups of sites had more taxa in common than did those towards the end of the axes. In Fig. 3 the taxa with extreme values for each of the variables can be identified. Invertebrates influenced by water hardness (several taxa at the right of Fig. 3) included the molluscs *Physella acuta* and *Potamopyrgus jenkinsi*; the crustaceans *Asellus aquaticus, Echinogammarus obtusidens*, and *Atyaephyra desmarestii*; the mayflies *Baetis pavidus, Caenis luctuosa, Cloeon cognatum*, and *Procloeon* sp.; the caddis *Hydropsyche exocellata*; the dragonflies *Orthetrum brunneum* and *Ischnura* sp.; and the beetles *Laccobius atrocephalus, L. sinuatus*, and *Laccophilus hyalinus*. In the lower right quadrant of Fig. 3 are the taxa most tolerant of organic pollution: the crustacean *Asellus aquaticus*, the mayfly *Baetis pavidus*, the mollusc *Physella acuta*, the midge *Chironomus* gr. *thummi*, tubificids, and the leech *Helobdella stagnalis*. Intolerant species (but typical of an intermediate degree of hard waters) occupied an upper-left position in Fig. 3 (the stonefly *Leuctra*

(Axis 1, partial correlation coefficient = −0.56, $p < 0.0001$; Axis 2, partial correlation coefficient = −0.06, $p = 0.64$; Axis 3, partial correlation coefficient = 0.29. $p = 0.02$: Axis 4, partial correlation coefficient = 0.06, $p = 0.61$). Axis 3 was highly correlated with altitude, distance from the spring source, and nitrites (Table 5); and, in the study area, the sites at higher altitudes and near the spring source coincided with less polluted reaches of the rivers.

## Discussion

Benthic macroinvertebrate assemblages along rivers have been the subject of much discussion since the River Continuum Concept, by Vannote et al. (1980), postulated the gradual replacement of the different species downstream in natural rivers. However, today very few rivers remain untouched, and sharp faunistic changes may be found as the result of sudden variation in water quality due to direct effect of humans, such as sewage input, flow regulation, and/or habitat destruction (Prat and Ward 1994). Hence, methods of water-quality bioassessment using benthic invertebrates, such as biotic indices (see Metcalfe 1989 for a review) and multivariate analyses, which classify and ordinate running-water sites (e.g., Wright et al. 1984, 1988, Leland et al. 1986, Bargos et al. 1990, Basaguren and Orive 1990, Gower et al. 1994), are increasingly used in the studies on river ecology.

Our study shows that variables indicative of water hardness, slope, flow, altitude, and nitrites are decisive in the distribution pattern of macroinvertebrate in the Genil River Basin. The 1st canonical axis, which explained the greatest part of the variance of the data, represented the longitudinal increase in water hardness and nutrient content, and was mainly indicative of degree of pollution. Nitrites constitute a variable clearly related to urban, industrial, and agricultural pollution. However, some variables measured in the water conductivity also come from these types of pollution, as stated in a previous paper in which potassium concentration (highly correlated also with canonical Axis 1) proved to be one of the main factors explaining the variance of the BMWP' biotic index (Zamora-Muñoz et al. 1995), because of its association with pollution by fertilizers and an olive-pressing residue (the main industrial waste in the study area). The 2nd axis was related mainly to water flow, and the 3rd and 4th axes were less significant. It has been observed elsewhere that rivers with a low level of pollution showed no correlation between ordination scores (by Correspondence Analysis) and BMWP biotic index due to the fact that, when the main environmental variables influencing the species distributions are other than pollution, values of this index do not change between sites (Bargos et al. 1990). In our study, a significant part of the variation in the data was explained by the BMWP' biotic index and the variables related to organic pollution.

On one hand, the characteristic fauna inhabiting the upper reaches of Sierra Nevada streams having steep slopes, rocky substrate, and soft waters (sites of TWINSPAN groups 26 and 27 in a lower left position in Fig. 2, with BMWP' values indicative of acceptable water quality) was separated from the fauna of Genil sites downstream of the Canales and Quéntar Reservoirs (sites of group 12 situated in an intermediate position in Fig. 2, with similar values of BMWP' values), as well as from headwater sites flowing over calcareous soil, with less hilly substrate and harder waters than the former (sites belonging to group 7, to the upper left position of Fig. 2, and with higher BMWP' mean values than the former groups). The differences in BMWP' mean values and taxa richness between headwater sites of calcareous rivers (TWINSPAN group 7) and those that flow over siliceous rock (TWINSPAN groups 26 and 27), may be explained by the lower productivity of Sierra Nevada streams (with the lower calcium content in the basin). On the other hand, groups of sites including middle and downstream reaches of rivers affected by organic pollution (to the right side of the diagram, with BMWP' values indicative of critical water quality) were isolated from sites in the rest of the basin. Most of the sites in the study area were polluted, i.e., highly enriched with nutrients, as indicated by the fact that the most frequent taxa were tolerant species.

The seasonal sorting of samplings from certain sites resulting from the TWINSPAN classification was probably due to seasonal pollution inputs at those sites, and their effects on faunal composition, rather than to invertebrate life histories. Most of those sites were polluted and are shown in the right side of the ordination diagram. In a previous paper on the seasonal dependence of the BMWP' index in the same

study area, it was found that the relationship between the index and seasonality was caused by pollution, and that low BMWP' values in some sites in winter could have been related to the olive-pressing residue dumped in some rivers (Zamora-Muñoz et al. 1995).

The headwater sites of the Genil River (rocky and hilly substrate, environmentally rather homogenous, colonized mainly by species of lotic-erosional habitats, with a high proportion of shredders) would correspond to heterotrophic upper reaches under the River Continuum Concept (Vannote et al. 1980). Downstream, below the Canales and Quéntar Reservoirs, streamflow was more constant than upstream, river bed and banks were more stable, and aquatic vegetation increased (Zamora-Muñoz 1992). These environmental conditions allow the establishment of lotic-depositional and lentic species (Ward and Stanford 1979), such as *Paraleptophlebia submarginata, Caenis luctuosa, Torleya major, Boyeria irene,* and *Gerris najas,* with an increase of grazers and collectors (molluscs, Baetidae, Caenidae, Hydropsychidae, Simuliidae), corresponding to the autotrophic region of middle reaches under the River Continuum Concept. This disturbance by river regulation may explain the position of these sites in the middle of CCA diagram, and also may explain why species richness at the sites downstream of Canales Reservoir was higher than at upstream sites (Intermediate Disturbance Hypothesis; Ward and Stanford 1983).

Downstream of the city of Granada, pollution levels were extremely high, causing local extinction of macroinvertebrates in several seasons (Zamora-Muñoz 1992); therefore these sites were eliminated from the analyses. Severe pollution affects macroinvertebrate groups more than single species (Hynes 1960), and, in those sites, only a few Diptera (*Eristalis* and *Psychoda*) and Tubificidae, the most tolerant species to organic pollution (Hynes 1960, Hellawell 1986), were occasionally found. From here to the Iznájar Reservoir, the river was in a recovery zone where the most frequent taxa were Tubificidae, Chironomidae (*Chironomus* gr. *thummi,* among them), *Baetis pavidus, Asellus aquaticus, Physella acuta,* and Erpobdellidae—all well-known tolerant taxa (Hynes 1960, González del Tánago and García de Jalón 1984, Hellawell 1986, Alba-Tercedor et al. 1991). Stoneflies, Heptageniidae, and case-bearing caddisflies (groups with many intolerant species) were absent or almost so, as reflected in the biotic index values obtained at those sites.

The similar results obtained in the multivariate analysis using family level identification, as with the BMWP' biotic index, and finer identification (species and genus) indicated that, in spite of the opinion of some authors (e.g., Resh and Unzicker 1975), family-level identification provides much information about water quality. However, to know the exact biological response to an environmental disturbance, succession, and greater or lesser tolerance of the organisms to certain alterations, we need a species-level identification (Furse et al. 1984), because the same family may contain species of different tolerances. It is noteworthy that the drop in the biotic index values of the groups from the left to the right of Fig. 2 is accompanied by a substitution of species from less to more pollution-tolerant (Fig. 3). Nevertheless even within the stoneflies, a group typically intolerant of organic enrichment of the waters (e.g., Hynes 1960, Hellawell 1986), the species *Nemoura fulviceps* had its optimal distribution in the middle of the biplot. This species belongs to Nemouridae, the stonefly family with the lower score in the BMWP' biotic index (Alba-Tercedor and Sánchez-Ortega 1988). In the caddisflies, *Hydropsyche instabilis,* which is found exclusively in non-polluted upper reaches of the basin (lower left of the biplot), is replaced by *H. exocellata* (lower right of the biplot), the most common species of this genus in the lower reaches of Iberian Peninsula rivers and the most tolerant to pollution (García de Jalón and González del Tánago 1986, Basaguren 1988, Basaguren and Orive 1990). Different degrees of tolerance were observed within the genus *Baetis,* with *B. muticus* and *B. maurus* showing the least tolerance, followed by *B. rhodani* and, the most tolerant, *B. pavidus,* as has been observed in other rivers (Alba-Tercedor et al. 1991, Zamora Muñoz et al. 1993, Alba-Tercedor et al. 1995).

The results obtained by the multivariate analyses were highly satisfactory for a correct interpretation of the assemblages and substitutions of macroinvertebrates, according to the main environmental variables measured in the basin. The range went clearly from clean to polluted sites, the main factors influencing the faunal distribution being the nutrient content and water hardness, which, besides the natural increase

downstream, appears to be due to pollutant input. Moreover, the classification of the sites according to the ecological characteristics of the taxa was very closely related to the classification given by the BMWP' biotic index, with respect to its water-quality significance, and according to the tolerance of the macroinvertebrate families to the pollution. Thus, we establish the usefulness of this index as an easy tool for water-quality assessment.

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APPENDIX 1. List of taxa from the study area after the elimination of rare taxa (those in <4 samples) with the abbreviations used in Fig. 3 in parentheses.

TURBELLARIA
  Tricladida
    Dugesiidae
      *Dugesia* gr. *lugubris* (DUGESIA)
    Planariidae
      *Polycelis felina* [Dalyell, 1814] (POLYCELI)
GASTROPODA
  Basommatophora
    Ancylidae
      *Ancylus fluviatilis* Müller, 1774 (ANCYLUS)
    Lymnaeidae
      *Lymnaea peregra* [Müller, 1774] (LPEREGRA)
    Physidae
      *Physella acuta* [Draparnaud, 1805] (PHYSELLA)
    Planorbidae
      *Planorbarius metidjensis* [Forbes, 1838] (PLANORBA)
  Prosobranchia
    Hydrobiidae
      *Potamopyrgus jenkinsi* [Smith, 1889] (POTAMOPY)
    Neritidae
      *Theodoxus fluviatillis* [Linnaeus, 1758] (THEODOXU)
    Thiaridae
      *Melanopsis dufouri* Férussac, 1823 (MELANOPS)
BIVALVIA
  Sphaeriidae
    *Pisidium casertanum* [Poli, 1791] (PISIDCAS)
OLIGOCHAETA
  Lumbricidae
    *Eiseniella tetrahedra* [Savigny, 1826] (EISENIEL)
  Lumbriculidae (LUMBRICU)
  Naididae
    *Stylaria lacustris* [Linnaeus, 1758] (STYLARIA)
    others (NAIDIDAE)
  Tubificidae (TUBIFICI)
ACHAETA
  Erpobdellidae (ERPOBDEL)
  Glossiphoniidae
    *Batracobdella paludosa*? [Carena, 1824] (BATRACOB)
    *Helobdella stagnalis* [Linnaeus, 1758] (HELOBDEL)
ARACHNIDAE
  Hydracarina (HYDRACAR)
CRUSTACEA
  Amphipoda
    Gammaridae
      *Echinogammarus obtusidens* Pinkster & Stock, 1972 (ECHOBTUS)
      *Echinogammarus simoni* [Chevreux, 1894] (ECHSIMON)
  Decapoda
    Astacidae
      *Procambarus clarkii* [Girard, 1852] (PROCAMBA)
    Atyidae
      *Atyaephyra desmarestii* [Millet, 1831] (ATYAEPHY)
  Isopoda
    Asellidae
      *Asellus aquaticus* [Linnaeus, 1758] (ASELLUS)
  Ostracoda
    Cypridae (CYPRIDAE)
INSECTA
  Ephemeroptera
    Baetidae
      *Beatis fuscatus* [Linnaeus, 1761] (BFUSCATU)
      *Baetis muurus* Kimmins, 1938 (BMAURUS)

*Baetis muticus* Linnaeus, 1758 (BMUTICUS)
*Baetis pavidus* Grandi, 1949 (BPAVIDUS)
*Baetis rhodani* Pictet, 1843–45 (BRHODANI)
*Centroptilum luteolum* [Müller, 1976] (CLUTEOLU)
*Centroptilum* gr. *pulchrum* (CPULCHRU)
*Cloeon cognatum* Stephens, 1835 (CLCOGNAT)
*Procloeon* sp. (PROCLOEO)
Caenidae
*Caenis luctuosa* [Burmeister, 1839] (CALUCTUO)
*Caenis pusilla* Navas, 1913 (CAPUSILL)
Ephemerellidae
*Ephemerella ignita* [Poda, 1761] (EIGNITA)
*Ephemerella maculocaudata* Ikonomov, 1961 (EMACULOC)
*Torleya* cf. *belgica* Leetage, 1917 (TORLEYA)
Ephemeridae
*Ephemera danica* Müller, 1764 (EPHEMERA)
Heptageniidae
*Ecdyonurus* sp. (ECDYONUR)
*Epeorus sylvicola/torrentium* (EPEORUS)
*Rhithrogena marcosi* Alba-Tercedor & Sowa, 1987 (RHMARCOS)
*Rhithrogena* gr. *semicolorata* (RHSEMICO)
Leptophlebiidae
*Habrophlebia eldae* Jacob & Sartori, 1984 (HABELDAE)
*Paraleptophlebia submarginata* [Stephens, 1835] (PARALEPT)
Oligoneuriidae
*Oligoneuriella marichuae* Alba-Tercedor, 1983 (OMARICHU)
*Oligoneuriella rhenana* [Imhoff, 1852] (ORHENANA)
Odonata
Aeshnidae
*Boyeria irene* [Fonscolombe, 1838] (BOYERIA)
Calopterygidae
*Calopteryx* sp. (CALOPTER)
Coenagrionidae
*Ischnura* sp. (ISCHNURA)
Gomphidae
*Onychogomphus uncatus* [Charpentier, 1840] (ONYUNCAT)
Libellulidae
*Orthetrum brunneum* [Fonscolombe, 1837] (ORBRUNNE)
Plecoptera
Leuctridae
*Leuctra fusca* [Linnaeus, 1758] (LEFUSCA)
Nemouridae
*Nemoura fulviceps* Klapalek, 1902 (NFULVICE)
*Protonemura meyeri* [Pictet, 1841] (PROMEYER)
Perlidae
*Dinocras cephalotes* [Curtis, 1827] (DINOCRAS)
*Perla marginata* [Panzer, 1799] (PERLAMAR)
Perlodidae
*Isoperla grammatica* [Poda, 1761] (ISOGRAMM)
Heteroptera
Corixidae
*Micronecta* spp. (MICRONEC)
Gerridae
*Gerris cinereus* [Puton, 1869] (GERCINER)
*Gerris najas* [De Geer, 1773] (GERNAJAS)
Hydrometridae
*Hydrometra stagnorum* [Linnaeus, 1758] (HYDROMET)
Nepidae
*Nepa cinerea* Linnaeus, 1758 (NEPACINE)
Coleoptera
Dryopidae
*Dryops gracilis* [Karsch, 1881] (DRYOPS)
*Helichus substriatus* [Müller, 1806] (HELICHUS)
Dytiscidae
*Agabus didymus* [Olivier, 1795] (ADIDYMUS)
*Deronectes fairmeirei* [Leprieur, 1876] (DFAIRMEI)
*Laccophilus hyalinus* [De Geer, 1774] (LACHYALI)
*Potamonectes clarcki* [Wollaston, 1962] (POTCLARC)
Dytiscidae larvae (LARVDYTI)
Elmidae
*Elmis maugetii* Latreille, 1798 (ELMIS)
*Limnius opacus* Müller, 1806 (LIMOPACU)
*Limnius volckmari* [Panzer, 1793] (LIMVOLCK)
*Limnius* spp. larvae (LARVLIMN)
*Riolus illiesi* [Steffan, 1958] (RIOLUS)
Gyrinidae

*Aulonogyrus striatus* [Fabricius, 1792] (AULONOGY)
*Orectichilus villosus* [Müller, 1776] (ORECTICH)
Gyrinidae larvae (LARVAS)
Haliplidae
*Haliplus lineatocollis* [Marsham, 1802] (HALIPLUS)
Helodidae (HELODIDA)
Hydraenidae
*Hydraena capta* Orchymont, 1936 (HCAPTA)
*Hydraena subdepressa* Rey, 1886 (HSUBDEPR)
Hydrophilidae
*Laccobius atrocephalus* Reitter, 1872 (LACATROC)
*Laccobius sinuatus* Motschulsky, 1849 (LACSINUA)
Hydrophilidae larvae (HYDROPLA)
Megaloptera
Sialidae
*Sialis nigripes* Pictet, 1865 (SIALIS)
Trichoptera
Brachycentridae
*Micrasema moestum* [Hagen, 1868] (MIMOESTU)
*Oligoplectrum maculatum* [Fourcroy, 1785] (OLIGOPLE)
Glossosomatidae
*Agapetus incertulus* McLachlan, 1884 (AGINCERT)
Hydropsychidae
*Cheumatopsyche lepida* [Pictet, 1834] (CHEUMATO)
*Hydropsyche brevis* Mosely, 1930 (HBREVIS)
*Hydropsyche exocellata* Dufour, 1841 (HEXOCELL)
*Hydropsyche infernalis* Schmid, 1952 (HINFERNA)
*Hydropsyche instabilis* [Curtis, 1834] (HINSTABI)
*Hydropsyche pellucidula* [Curtis, 1834] (HPELLUCI)
*Hydropsyche* cf. *punica* Malicky, 1981 (HCFPUNIC)
Hydroptilidae
*Hydroptila vectis* Curtis, 1834 (HYDROPTI)
Lepidostomatidae
*Lasiocephala basalis* [Kölenati, 1848] (LASIOCEP)
Leptoceridae
*Athripsodes* sp. (ATHRIPSO)
Rhyacophilidae
*Rhyacophila munda* McLachlan, 1862 (RHMUNDA)
*Rhyacophila nevada* Schmid, 1952 (RHNEVADA)
Sericostomatidae
*Sericostoma baeticum/vittatum* (SERICOST)
Polycentropodidae
*Polycentropus kingi* McLachlan, 1881 (POLYCENT)
Diptera
Anthomyidae
*Limnophora* sp. (LIMNOPHO)
Athericidae
*Atherix* sp. (ATHERIX)
Ceratopogonidae
Ceratopogoninae (CERATOPO)
Chironomidae
Chironominae (CHIRONOM)
*Chironomus* gr. *thummi* (CHIROTHU)
*Corynoneura* sp. (CORYNONE)
Orthocladinae/Diamesinae (ORTHOCLA)
Tanypodinae (TANYPODI)
Tanytarsini (TANYTARS)
Culicidae
Culicinae (CULICINA)
Dixidae
*Dixa* sp. (DIXA)
Empididae
Clinocerinae (CLINOCER)
Ephydridae
Ephydrinae (EPHYDRIN)
Limoniidae
*Antocha* sp. (ANTOCHA)
*Dicranota* sp. (DICRANOT)
*Hexatoma* sp. (HEXATOMA)
Tabanidae
*Tabanus* sp. (TABANUS)
Tipulidae
*Tipula* sp. (TIPULA)
Psychodidae (PSYCHODI)
Simuliidae (SIMULIID)
Stratiomyidae (STRATIOM)