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THE EFFECTS OF A BASIC EFFLUENT ON MACROINVERTEBRATE COMMUNITY STRUCTURE IN A TEMPORARY MEDITERRANEAN RIVER

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Abstract

Macroinvertebrate communities and environmental variables were assessed seasonally for 1 year in a temporary river in South Portugal receiving an effluent with high conductivity, pH, sulphates, nitrates and low oxygen content. The usefulness of the ordination method canonical correspondence analysis (CCA) and the classification method cluster analysis (UPGMA) were examined to evaluate the perturbation. Macroinvertebrate samples were segregated along the first ordination axis by CCA, which in turn correlated with sulphates and nitrates. CCA produced a two-dimensional distribution of sites similar to the grouping formed by cluster analysis. In general, three or four groups were distinguished. Immediately downstream of the effluent discharge point, only taxa tolerant to low oxygen, high pH and high sulphate and nitrate concentrations were present. Further downstream, sites had a community similar to the reference sampling locations. During flowing conditions the CCA ordination axis 1 was also correlated with several classic measures of water quality (i.e. taxon richness, diversity and biotic indices). In other periods, only the percentage of Ephemeroptera, Plecoptera and Trichoptera (%EPT) and the ratio EPT/(Chironomidae + EPT) were significantly correlated with CCA axis one. This suggests that ordination methods outperform benthic indices in detecting pollution during low flows and segregated polluted from clean/recovered sites in all periods. © 1997 Elsevier Science Ltd. All rights reserved

Keywords: Temporary rivers, multivariate analysis, alkaline pollution, macroinvertebrates, bioindicators.

INTRODUCTION

The south of the Iberian Peninsula is characterized by a Mediterranean climate with hot dry summers and mild winters. This results in intermittent flow in many rivers, with the systems shifting between lotic and lentic conditions during the year (Pinto, 1994; Morais, 1995). As a consequence, there is a considerable dynamism in other environmental parameters (suspended solids, hardness, conductivity, silica, etc.), major energy sources and in the dominance in major algal, macroinvertebrate and macrophyte groups (Pinto, 1994; Morais, 1995). Therefore, seasonal factors related to the hydrological cycle are the major determinants in community structure and function. These aspects contrast with rivers from north and central Portugal, where the longitudinal gradients are more important in controlling the macroinvertebrate communities (Cortes, 1989, 1992; Graça *et al.*, 1989).

Although the Mediterranean streams are located in or near areas of growing water demand for tourism and agriculture, they have been poorly studied, especially in south Portugal. Their biology under pollution stress is particularly poorly documented.

Biological methods based on aquatic macroinvertebrates have been widely used to assess general water quality in rivers. At its simplest form, changes in water quality are equated to changes in diversity or biotic indices (for reviews, see Washington, 1984; Metcalfe, 1989).

One aspect of the water pollution widely investigated in freshwaters is pH, since acid rain and acid mine drainage are major perturbing factors in industrialized countries. Decreases in pH have been related to a general decrease in diversity of aquatic systems (Winner *et al.*, 1980; Feldman & Connor, 1992). In contrast, the effects of high pH effluents in rivers have not been well documented. This paper assesses the effect of an industrial basic effluent in the structure of macroinvertebrate communities in a Mediterranean intermittent river of south Portugal (Alentejo). Multivariate methods have been widely used to evaluate changes in water quality (Richards *et al.*, 1993; Rossaro & Pietrangelo, 1993; Gower *et al.*, 1994; Castella *et al.*, 1995). In this study we used canonical correspondence analysis (CCA) (ter Braak, 1988) and cluster analysis (UPGMA) (Boesch, 1977; Legendre & Legendre, 1979).

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MATERIAL AND METHODS

The study area was located at Alentejo, south Portugal. The only source of pollution in the river was an industrial effluent with high conductivity, high content in sulphates and nitrates and high pH. Due to the lack of superficial flow during summer, water at the discharging point was 100% effluent. Samples of macroinvertebrates and water were taken seasonally in eight sites downstream from the effluent output (P0···P7) and eight reference sites (R1···R6), two of which were located in a nearby river (RC1 and RC2) in order to account for downstream trends. The distance between the first (R1) and last (P7) site was 45 km. Sites R1, R2, R6 and P5 were not sampled in summer and autumn because they were dry.

Biological samples were taken in winter (February), spring (May), summer (September) and autumn (November) of 1994 with a hand net (30×30 cm aperture and 0.5 mm mesh size) in a kick and sweep way. Each sample was composed of six sub-samples taken in the major macrohabitats. Organisms were sorted alive and preserved in 70% ethanol for further identification to the highest possible taxonomic level. At each sampling period, water temperature and oxygen were measured in the field with a portable oxygen-meter (WPW/OXI196). Water samples were collected for further chemical analysis: pH and conductivity (specific meters), sulphates, nitrates, phosphates, copper and iron (APHA, 1980). Samples were transported in a cold box (4°C) and analysed within 24 h.

Biological assemblages were ordinated by CCA using the program CANOCO (ter Braak, 1988) after a log transformation $[\ln(x+1)]$. The option of downweighting rare taxa was applied. CCA examines variations in the community composition by constraining ordination axes to be linear combinations of environmental variables. In the ordination diagram, environmental variables are represented by arrows pointing in the direction of maximum change and the arrow length indicates the importance of the environmental variable (Palmer, 1993; Gower *et al.*, 1994). Weighted intra-set correlation coefficients were used to identify ecological explanations of the ordination axes derived from the biological data.

As a classification method, cluster analysis (Legendre & Legendre, 1979) was applied to the same data to extract discrete groups. The distances were calculated by an unweighted average linked procedure (UPGMA). Resemblance among data sets was calculated using the Bray–Curtis dissimilarity method, which has the desirable property of not depending on joint absences of species in the samples (Boesch, 1977).

Several methods for the assessment of water quality were also applied: taxon richness (S), Shannon–Wiener diversity index (H′), Belgium Biotic Index (BBI; De Pauw & Vanhooren, 1983), Biological Monitoring Working Party Score system adapted to the Iberian Peninsula (BMWP′; Alba-Tercedor & Sánchez-Ortega, 1988), Average Score Per Taxon (Armitage *et al.*, 1983) derived from BMWP′ (ASPT′), percentage abundance of Chironomidae (% Chir) and percentage of Ephemeroptera–Plecoptera–Trichoptera (%EPT) and ratio of EPT/(Chir + EPT) (Klemm *et al.*, 1990).

The ecological meaning of the first two axes of CCA ordination was assessed through Spearman rank correlations with the previous biological methods.

RESULTS

General

In general, the effluent discharge caused a significant decrease in the dissolved oxygen of the river water ($P \leq 0.05$, except in spring; Mann–Whitney U test) and a significant increase in conductivity, sulphates and nitrates ($P \leq 0.05$; Mann–Whitney U test) (Table 1). The pH was strongly affected but only near the effluent output (sites P0 and P1).

A total of 117 macroinvertebrate taxa were recorded. The most abundant taxa during the year were Chironomidae (Diptera) followed by *Caenis luctuosa*, *Baetidae* (Ephemeroptera), *Physa acuta* (Gastropoda) and *Atyaephyra desmarestii* (Decapoda). Some taxa were exclusively found in lotic conditions, whereas others were exclusively lentic. The Plecoptera (several species), *Ecdyonurus aurantiacus*, *Siphlonurus* sp., *Ephemerella* sp., (Ephemeroptera), cased Trichoptera and *Oulimnius rivularis* (Coleoptera) are examples of the former group, whereas *Anax imperator*, *Gomphus pulchellus*, *Orthetrum coerulescens* (Odonata), *Berosus affinis* (Coleoptera), *Chironomus thummi/plumosus* group and Culicidae (Diptera) are examples of taxa occurring almost exclusively during lentic conditions.

In the reference sites, Plecoptera (only in winter), *Caenis luctuosa*, Baetidae (Ephemeropera), Hydracarina, *Oulimnius rivularis* (Coleoptera) and *Atyaephyra desmarestii* (Decapoda) were abundant. In the sites immediately below the effluent output, most of these taxa were replaced by Chironomidae, Culicidae (Diptera) and *Berosus affinis* (Coleoptera). These and other taxa are able to use atmospheric oxygen (e.g. Culicidae) or are tolerant to hypoxia conditions (e.g. *Chironomus thummi/plumosus* group).

CCA ordination

Although all nine environmental parameters were included the early CCA ordinations, those variables with high variance inflation factors (VIF > 20, indicating strong multicollinearity: the case of DO mg litre^{-1} and conductivity) or negligible variances (phosphates) were eliminated from the analyses.

For all sampling periods, CCA site ordination segregated along the first axis reference sites (Group A) from the other sites (Fig. 1). This segregation was related to oxygen (high in reference sites) and nitrates and sulphates (high in sites located downstream of the effluent output). In general, the second axis segregated sites located immediately below the effluent output

(Group B, high pH) from the others. This can also be confirmed by the higher value of intra-set correlation coefficients for nitrates and sulphates with axis 1 (Table 2), which means that this axis depends essentially of both variables.

In all CCA ordinations, the Monte Carlo test showed that the extracted axis 1 was statistically significant ($p \leq 0.03$) when compared with a random (99 permutations) null pattern of species groups.

Cluster analysis

For all sampling periods, cluster analysis segregated reference sites from sites below the effluent output (Fig. 2). In some occasions, the sites located far away from the effluent output were clustered with the reference sites (e.g. P7). The site where the effluent is discharged was generally segregated from the others, and the same occurred with the two sites immediately below.

Biological indices and ordinations techniques

In general, CCA ordinations identified sulphates, nitrates and dissolved oxygen as the chemical variables most highly correlated with axis 1, indicating a strong pollution gradient along this axis and pH as the most highly correlated with axis 2. Therefore, these axes were

Table 1. Changes in the physico-chemical parameters of the stream caused by the effluent discharge

Parameter	Season	Ref[a]		DS[b]		*U*[c]	PO[d]
		Mean	Range	Mean	Range		
Temperature (°C)	Winter	12.6	12.0–13.6	12.5	12.2–12.8	28	12.4
	Spring	14.1	12.0–16.5	13.1	11.9–14.0	27.5	14.0
	Summer	22.9	20.7–24.8	21.5	203–23.3	18	21.3
	Autumn	—	—	—	—	—	—
Oxygen (%sat)	Winter	99	95–101	50	71–86	40**	73
	Spring	86	74–105	69	36–95	28.5	75
	Summer	83	71–96	57	36–75	24*	36
	Autumn	122	100–145	62	36–93	25**	71
Dissolved oxygen (mg litre^{-1})	Winter	13.0	12.6–14.2	10.7	9.6–12.0	40**	9.7
	Spring	8.6	7.7–10.4	7.0	3.6–9.6	25.5	8.0
	Summer	9.4	7.9–10.2	6.5	4.3–8.0	24*	4.3
	Autumn	—	—	—	—	—	—
pH	Winter	7.6	7.2–8.5	8.5	7.9–9.5	36.5*	9.5
	Spring	7.8	6.7–8.6	8.5	6.8–11.3	21	11.3
	Summer	8.5	8.2–8.6	7.4	7.2–8.9	20	8.9
	Autumn	8.5	8.0–8.8	8.4	6.8–11.2	15	11.2
Conductivity (μS cm^{-1})	Winter	274	216–471	606	511–769	40**	769
	Spring	412	312–552	2380	2180–2520	40**	22480
	Summer	1510	800–1890	4580	3560–5090	25**	3580
	Autumn	1550	932–2190	3770	3610–4070	25**	3960
Sulphates (mg litre^{-1})	Winter	29	24–50	131	96–188	40**	188
	Spring	28	23–46	756	700–830	40**	830
	Summer	31	9–74	1210	870–1520	25**	870
	Autumn	85	42–144	1000	963–1060	25**	973
Nitrates (mg litre^{-1})	Winter	0.3	0.1–0.9	10	6.9–12.2	40**	10.2
	Spring	0.2	constant	83	43–129	40**	129
	Summer	0.3	0.2–0.4	135	110–160	25**	150
	Autumn	0.3	0.2–0.4	172	163–191	25**	166
Phosphates (mg litre^{-1})	Winter	0.1	0.1–0.2	0.2	0.1–0.2	29	0.1
	Spring	0.2	0.1–0.3	0.3	0.1–0.7	26.5	0.1
	Summer	0.4	0.3–0.6	0.2	0.1–0.2	25**	0.2
	Autumn	0.2	0.1–0.3	0.2	0.1–0.11	16.5	0.1
Iron (mg litre^{-1})	Winter	0.31	0.03–1.23	0.11	0.04–0.33	24.5	0.04
	Spring	0.28	0.12–0.60	0.05	0.02–0.14	37*	0.03
	Summer	0.37	0.27–0.55	0.21	0.06–0.40	22	0.06
	Autumn	0.40	0.12–1.16	0.06	0.06–0.07	25**	0.06

[a] Reference sites ($n = 8$ in Winter and Spring, $n = 5$ in Summer and Autumn).
[b] Downstream sites ($n = 5$).
[c] Mann–Whitney *U*-test.
[d] Site immediately downstream of the effluent output.
*$p \leq 0.05$; ** $p \leq 0.01$.

used to assess (through Spearman rank correlations) the relative effectiveness of the biological method (Table 3).

All the indices were significantly correlated with CCA axis 1 in winter. For other periods only %Chir (spring), %EPT and the ratio EPT/(Chir + EPT) were highly correlated with axis 1. This suggests that indices such as BBI, BMWP′ and ASPT′ are useless when the conditions are clearly lentic.

DISCUSSION

Although in natural conditions the studied river had no flow during summer and autumn, the effluent was responsible for a certain continuity between pools located downstream. These pools varied in size between dozens and several hundreds of square metres, and from some centimetres to + 1.5 m deep. As previously reported for other temporary rivers (e.g. Williams, 1987; Morais, 1995), the environmental conditions were highly variable through the year. This suggests broad tolerance limits for most of the taxa permanently living in the river and/or species replacement through time.

Among the permanent living taxa, there were several Gastropods (e.g. *Physa acuta*, *Lymnaea peregra*) and the ephemeropteran *Caenis luctuosa*. These were abundant and their broad tolerance limits have been referred to elsewhere (e.g. Cortes, 1989). However, the

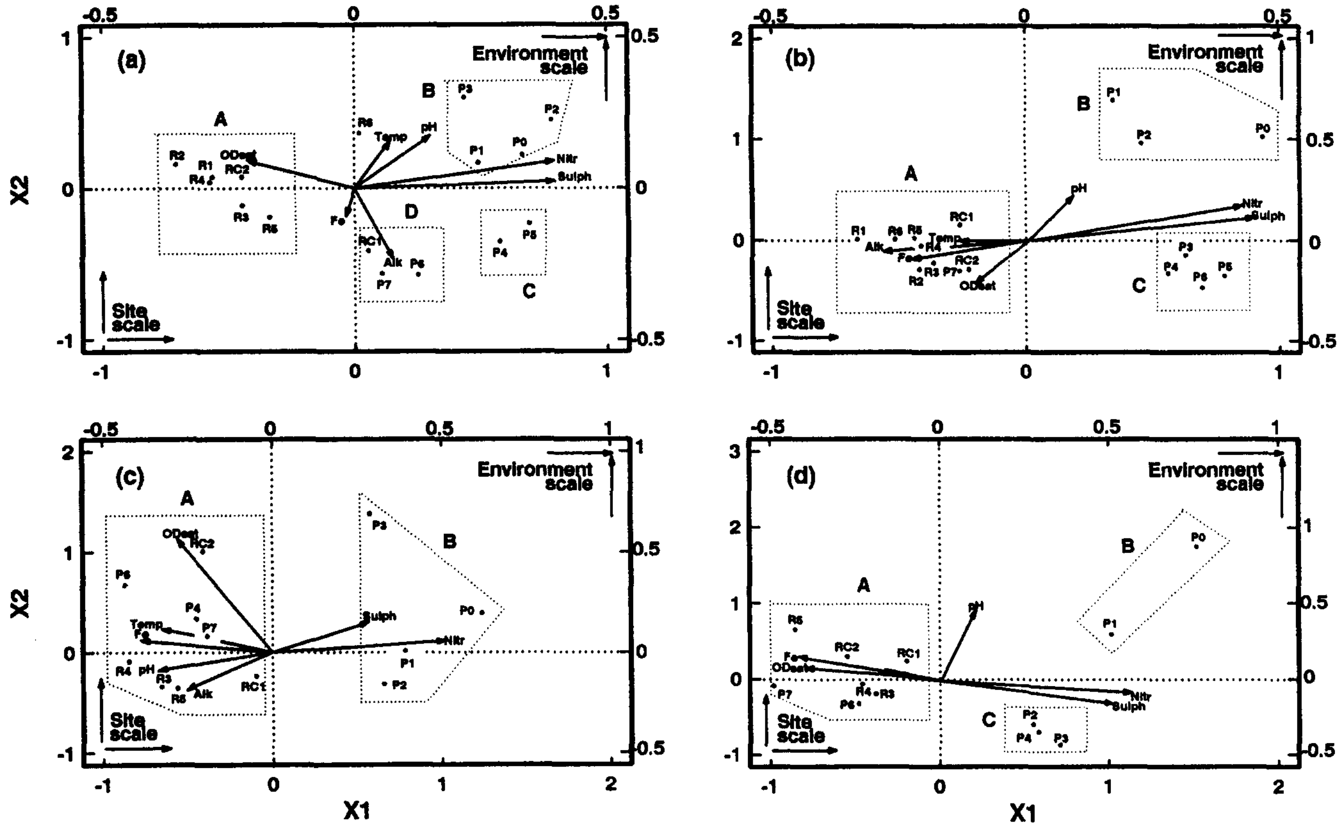

Fig. 1. Site-environment biplots based on CCA axes 1 and 2: (a) winter, (b) spring, (c) summer, (d) autumn. Temp., temperature; Nitr., nitrates; Sulph., sulphates; ODsat., oxygen; Alk., alkalinity; Fe, iron.

Table 2. Eigenvalues, percentage of variance explained by the first two axes (weighted intra-set correlation between CCA axes 1 and 2 and environmental variables)

Environmental variable	Winter		Spring		Summer		Autumn	
	X1	X2	X1	X2	X1	X2	X1	X2
Temperature	0.172	0.507*	−0.319	0.032	−0.665**	0.295	—	—
DO(% sat)	−0.509*	−0.282	−0.189	−0.609**	−0.474	0.142	−0.671**	0.082
pH	0.344	0.529*	−0.187	−0.681**	−0.651**	−0.318	0.168	0.932***
Sulphates	0.917***	0.075	0.916***	0.323	0.535*	0.415	0.826***	−0.271
Nitrates	0.881***	0.269	0.879***	0.348	0.939***	0.061	0.910***	−0.280
Iron	−0.029	−0.266	−0.477	−0.199	−0.680**	0.225	−0.669**	0.280
Eigenvalues	0.202	0.113	0.251	0.139	0.322	0.172	0.428	0.267
% variance	51.2		53.8		53.3		55.6	

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

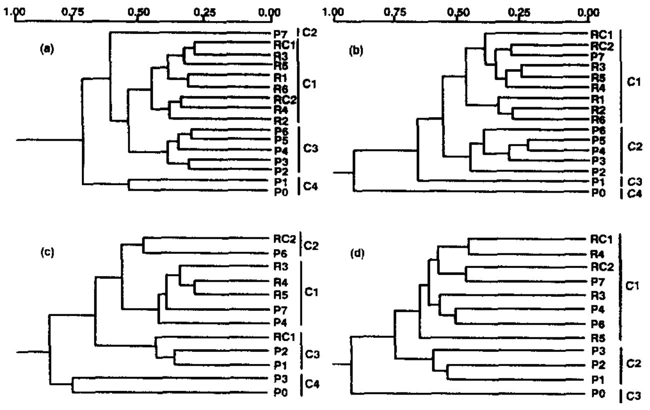

Fig. 2. Cluster analysis: (a) winter, (b) spring, (c) summer and (d) autumn.

vast majority of stream invertebrates were insects which may abandon the water when the conditions deteriorate (e.g. several Heteroptera and Coleoptera). Other taxa were present only on particular occasions; this was the case of Plecoptera (*Nemoura* sp., *Capnia bifrons*, *Tyrrenoleuctra* sp., *Hemimelaena flaviventis*, *Isogenus franzi* and *Isoperla* sp.) and several Ephemeroptera (*Ecdyonurus aurantiacus*, *Siphlonurus* sp., *Ephemerella* cf. *ignita*, *Habrophlebia* sp. and *Ephoron virgo*) which were present only during flowing conditions. All these species have a multivoltine life-cycle which allows the colonization of variable environments. This same observation on seasonality of the same taxa has already been referred for other temporary rivers in south Portugal (Pinto, 1994; Morais, 1995).

Some taxa present only in lentic periods were *Sympetrum foscolombei*, *Anax* spp., *Crocothermis servilia*, *Trithemis annullata* (Odonata), *Notonecta* spp., *Hydrometra stagnorum* (Heteroptera), *Ochthebius* sp., *Coelambus* sp., *Colymbetes* sp. (Coleoptera) and *Ecnomus* sp. (Trichoptera). In general, these taxa have also been already reported for nearby temporary rivers (Morais, 1995) or for summer samples in other regions (Landa, 1968).

The summer and autumn communities (lentic period) had a large proportion of atmospheric air breathing organisms (many Coleoptera and Heteroptera, some Diptera) or taxa able to resist low oxygen concentrations (e.g. *Chironomus thummi/plumosus* group; Paine & Gaufin, 1956). The low oxygen stress may be a natural event. The poor riparian vegetation of rivers in the region allows abundant light to reach the stream bed. Submerged plants (e.g. *Myriophyllum* sp. and *Ceratophyllum* spp.) become very abundant and may fill the pools. In summer, many aquatic plants in this region decay (Ferreira, 1992). This abundance of organic matter, zero flow and high temperatures (occasionally up to 40°C in the air) may favour a high microbial activity resulting in anoxia (< 2 mg litre^{-1}) conditions.

The above reason may explain why, in summer, the oxygen was not correlated with any of the ordination axes. On the other hand, summer cluster analysis aggregated two polluted sites (P1, P2) and one reference site (RC1). This may be explained by the intensity of the natural stressing conditions. With the advance of the dry season, ponds become smaller, with increase in salinity due to evaporation, accumulation of plant detritus and increase in temperature. The conditions may become harsh and, in some way, similar to those observed in the polluted sites, with a predominance of very tolerant taxa. These reasons explain why the biotic indices failed to discriminate between unpolluted and polluted sites. Although many biotic indices have been used to evaluate the general condition of waters (e.g. BBI—De Pauw & Vanhooren, 1983; BMWP and ASPT—Armitage *et al.*, 1983), they strongly rely on intolerance to low oxygen content or taxa with narrow tolerance limits. Therefore, the actual biotic indices seem to be useless to assess water quality in the temporary Mediterranean rivers when superficial flow is absent.

The apparent shift in biological conditions during the year justifies a separated data treatment for all sampling periods, decreasing the noise caused by the natural seasonal variation.

CCA ordination showed that the main disturbing effects of the effluent relied on the high levels of sulphate and nitrate (which were highly correlated with conductivity) and high pH, affecting not only the structure of the benthic communities, but probably also the

Table 3. Spearman rank correlation coefficients between CCA axes 1 and 2 and the biological indicators of water quality

Biological indicator[a]	Winter		Spring		Summer		Autumn	
	X1	X2	X1	X2	X1	X2	X1	X2
S	−0.560*	−0.030	−0.300	−0.074	0.170	−0.459	−0.071	−0.049
H′	−0.618*	0.112	−0.038	−0.138	0.077	−0.364	−0.070	0.490
BBI	−0.763**	−0.217	−0.203	−0.370	0.151	−0.583	−0.278	−0.244
BMWP′	−0.809**	−0.065	−0.021	−0.412	0.014	−0.587	0.098	−0.336
ASPT′	−0.709**	−0.285	−0.415	−0.703**	−0.294	−0.168	−0.277	−0.487
% Chir	0.794**	0.285	0.791**	−0.315	0.336	0.323	0.455	0.196
%EPT	−0.658**	−0.197	0.165	0.697**	−0.711	−0.185	−0.760*	−0.252
EPP(Chir + EPT)	−0.784**	−0.225	−0.603	−0.352	−0.556	−0.597*	−0.788**	−0.329

[a] Defined in the Materials and Methods section.
* $p \leq 0.05$; ** $p \leq 0.01$.

production. This disturbing effect was corroborated by cluster analysis.

Unlike sulphates, the nitrates, pH and oxygen approached values of reference sites in the lower sector of the river. Here, according to CCA, and cluster analysis, the biological communities were similar to the ones observed in reference sites, suggesting some recuperation.

In the 'recovered' communities, taxa such as Simuliidae (Diptera) and *Hydropsyche* spp. (Trichoptera) were abundant. These taxa feed on fine particles carried by the water, including algae (Tachet *et al.*, 1987; Morais, 1995), and their increase may be related to an increase in the periphyton and phytoplankton, favoured by the high nitrogen concentrations. Previous research in nearby rivers has shown that they are limited by nitrogen (Morais, 1995). If this is the case, the effluent is also causing eutrophication in the river and stimulating primary production. Gut content analyses have shown that algae are very important in the diet of these filter feeders in the region (Morais, 1995).

Finally, species richness and diversity indices have been used as indicators of water quality (e.g Barton & Metcalfe-Smith, 1992; Gower *et al.*, 1994). The application of diversity indices is based on the assumption that favourable environments have more species which gradually are replaced by an increase in the number of individuals in the remaining species, as the conditions become more adverse (e.g. Wilhm & Dorris, 1968). However, many studies have failed to relate it with environmental stress (e.g. Godfrey, 1978; Kansanen *et al.*, 1990). In the present study it was found that, although the effluent generally caused a decrease in taxa richness, the effect was very localized and, in some seasons, species richness had a maximum value in some of the polluted sites (P3, P4). Consequently, the multivariate approach showed that species replacement is more important than changes in species richness in biomonitoring programs.

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