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Factors Affecting Macroinvertebrate Richness and Diversity in Portuguese Streams: a Two-Scale Analysis

key words: macroinvertebrates, taxa richness, diversity, organic matter, substratum

Abstract

We analysed the spatial patterns in macroinvertebrate taxon richness and abundance at two scales: sampling unit and basin. We sampled 12 stream sites in three zones of Portugal, differing in climate geomorphology and water chemistry. At a sampling unit scale, substratum organic matter content, depth and the dominant size of substratum particles were correlated with numbers of taxa and individuals. We propose that the number of taxa at a small scale depends on the number of individuals, which in turn is the result of organic matter accumulation, hydrologic and substratum characteristics. The environmental parameters better explaining the large-scale biological data were temperature, minimum size of substratum particles and pH. Regardless of the relative importance of variable types and mechanisms regulating stream invertebrates along the climatic gradient, rivers from the North and Centre appeared to be richer in taxa than the typically Mediterranean streams in the South.

1. Introduction

One important attribute of communities is their species richness and diversity. Various mechanisms have been indicated as controlling species diversity, including productivity, habitat heterogeneity and biotic interactions (TOWNSEND, 1989; TOWNSEND *et al.*, 2002). Rivers and their adjacent riparian zones are considered to be dynamic, complex and diverse systems (RISSER, 1990; NAIMAN *et al.*, 1993). Benthic macroinvertebrates are an important component of the river biota. At a large scale, diversity of invertebrates along and among rivers is affected by factors such as longitudinal zonation of river abiotic conditions (VANNOTE *et al.*, 1980; CLENAGHAN *et al.*, 1998), channel width and catchment size (MALMQVIST and HOFFSTEN, 2000), discharge (CORTES *et al.*, 2002), temperature and pH (TOWNSEND *et al.*, 1983; CLARKE and SCRUTON, 1997).

At a stream reach scale, invertebrates have, in general, a clumped distribution, which is assumed to be related to the mosaic of interchanging conditions in substratum, flow conditions, depth and many others (TOWNSEND, 1989; CORTES *et al.*, 2002). These conditions are likely to change at a scale of only a few metres or centimetres. At this small scale, factors indicated as regulating macroinvertebrate distribution and richness are current velocity, substratum particle size (MARCHANT *et al.*, 1985; WILLIAMS and MOORE, 1986; ARUNACHALAM *et al.*, 1991), substratum stability (MALMQVIST and OTTO, 1987), organic matter in the

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substratum (WILLIAMS and MOORE, 1986), the spatial heterogeneity of the habitat (DOWNES *et al.* 1995) and sediment grain size and heterogeneity (WILLIAMS, 1980; GAYRAUD and PHILIPPE, 2001).

The relative importance of factors affecting the diversity of aquatic macroinvertebrates differs among studies, suggesting there is considerable inter-habitat and inter-climatic variation. It is therefore important to document factors correlated with invertebrate diversity, for a range of habitats and climates. The objective of the present study was to evaluate how macroinvertebrate richness is affected at two scales, the stream-reach scale, and the between-catchment scale by factors such as climate and water chemistry. Our sample size was 693 Surber samples distributed among 12 rivers located in three areas along a climatic gradient from North Portugal, with an Atlantic climate characterized by comparatively low temperature and high rainfall, to South Portugal, with a typical Mediterranean climate, and lower rainfall but with greater variability inducing a temporary regime and surface flow interruption during the summer dry period.

Since across – systems diversity has been related to the complexity of the habitat, overall production, and the predictability, or variability of environmental conditions, we predicted that at a small scale, the number of taxa would be a function of substratum heterogeneity (= habitat complexity) and organic matter availability (food resource). We also predict that at a large scale, Mediterranean streams would have a reduced number of taxa due to higher ecological constraints resulting from environmental instability.

2. Methods

2.1. Study Area

We selected 3 areas in Portugal along a temperature and precipitation gradient (Fig. 1). Precipitation conditions were similar in the North and Centre and comparatively higher than in the South (Table 1). Rivers in the North run through deep valleys in mountain areas, whereas in the South they run through relatively flat landscape. From each area we selected similar river sites in terms of pollution free conditions, width and presence of trees in the riparian zone, although they were less dense in South.

Table 1 Range of values for several climatic parameters in the investigated stream basins of North, Centre and South Portugal. Cartographic source of data http://195.22.0.189/atlas/c_escoamento.html.

Parameter	North	Centre	South
Runoff (mm)	200–800	600–1000	25–50
Evapotranspiration (mm)	450–700	600–800	<500
Annual precipitation (mm)	700–1600	1200–1600	<500
Number of days with precipitation	75–100	75–>100	50–75
Air temperature (°C)	7.5–15	10–16	>16
Total hardness (mg L ⁻¹ CaCO ₃)	0–50	0–50	200–500

In the North, the rivers were the Olo (N1), Pinhão (N2), Sordo (N3) and Tanha (N4), draining the Douro catchment. They are second and third order rivers, draining granite catchments; therefore, their waters were acidic and had low concentrations of inorganic compounds. Sampling sites ranged from 460 m (N4) to 990 m (N1) a.s.l. In Central Portugal the rivers were the Agadão (C1), Ladeiras (C2) (Serra do Caramulo, Vouga basin), S. João (C3) and Sotão (C4) (Serra da Lousã, Mondego basin). They were third to fifth order rivers, running through forested areas (mainly *Pinus* spp. and *Eucalyptus globulus*) with a schistose substratum. In terms of altitude, sampling sites ranged from 150 m (C1) to 340 m (C4) a.s.l. In the South, we sampled the rivers Ardila (S1), Degebe (S2) Vascão (S3) and Xévorá

Figure 1. Location of the 12 studied rivers in Portugal.

(S4), all of which are in the Guadiana river basin. The rivers flow through siliceous and low elevation areas (<100 m) and typically have a temporary flow regime, with flow being interrupted for variable periods, but generally 3 months in a year.

2.2. Invertebrate Sampling

Macroinvertebrates were sampled in June and October (North), April and September (Centre and South) 1996. Sampling coincided with the end of periods of high (late spring) and low (early autumn) precipitation. At each location, we took samples along 5 transects perpendicular to the river margin, 6 samples per transect ($6 \times 5 = 30$ samples/site). The distance between transects was ca. 4 metres. This gave $30 \text{ samples} \times 12 \text{ rivers} \times 2 \text{ seasons} = 720$ samples in theory. However, the number of samples actually obtained was 693 due to size constraints limiting sampling in some rivers. Samples were taken with a Surber sampler (0.1 m^2 ; $500 \mu\text{m}$ mesh size). We collected samples by perturbation of the substratum by hand to a depth of approximately 8 cm. Samples were preserved in the field with 4%

formaldehyde. In the laboratory the samples were washed through a series 5-mm, 2-mm, 1-mm and 0.5-mm sieves, the animals were sorted in white trays, preserved in 70% ethanol and later identified to genus or family (Oligochaeta and most Diptera) levels. Hydracarina were not identified further.

2.3. Environmental Factors

When taking Surber samples, we measured the size of the substratum particles at 8 points within the Surber frame (each corner and mid-way between corners). Substratum particles were measured second longest axis and classified into size classes: 1 (<0.5 mm), 2 (0.5–2 mm), 3 (2 mm–1 cm), 4 (1–5 cm), 5 (5–10 cm), 6 (10–20 cm) and 7 (> 20 cm). For each Surber sample we also recorded the depth and the current velocity at 0.6 depth using current meters. Discharge was computed by the integration of depth, width and current velocity measurements taken in a cross section of stream with homogeneous conditions (ALLAN, 1995). Dissolved oxygen, pH and conductivity were measured with field meters.

One litre of stream water was collected from each site and transported to the laboratory for determination of alkalinity (APHA 1995). AFDM of suspended organic matter was measured by filtering 1 litre of stream water through a pre-weighed membrane filter (1.2 µm). The filter was dried at 50 °C for 48 hours, and burnt at 500 °C for 5 hours.

In rivers from the Centre and South, the mass of the biofilm attached to the substratum was measured by taking a submerged stone located a few centimetres upstream of each Surber sample site. An area of 7.5 cm² was delimited using a plastic cap and the biofilm scraped with a razor blade into an Eppendorff vial (2 ml). Vials were transported in ice chests to the laboratory and the AFDM was obtained as the difference between dry mass (50 °C for 48 hours) and ash (500 °C for 5 hours).

In rivers from the North and Centre, coarse organic matter in each Surber sample was also estimated after removal of invertebrates and the mineral fraction. The organic fraction was dried to constant mass (up to 5 days at 50 °C), ignited (500 °C, 5 hours) and AFDM determined to the nearest 0.1 g.

2.4. Statistical Treatments

The number of taxa in each Surber sample was taken as an indicator of richness. Since the number of taxa is strongly dependent on sample size (number of specimens) we also estimated rarefied richness (Primer software, CLARKE and WARWICK, 2001) corresponding to the expected number of taxa in a sample of 40 individuals. Rarefied richness is expressed as ES(40). Samples with < 40 individuals were not taken into account for the computation of rarefied richness. They account for 19%, 10% and 30% of the samples for rivers in the North, Centre and South, respectively.

The effect of environmental factors on number of taxa (S), number of individuals (N) and rarefied richness (ES(40)) was assessed by computing multiple rank correlations (Spearman coefficient) with the variables measured at the location where each Surber sample was collected. Habitat complexity was assessed in several ways: (1) size of the dominant substratum particles (Gmode), smallest (Gmin) and largest (Gmax) substratum particles and the number of different substratum size classes.

To compare the three geographic zones, we pooled Surber samples from each stream and date and computed the total number of taxa, the Shannon diversity index H' and an index of species richness, $S/\sqrt{N}$ (Menhinick's index; HELLAWELL, 1978) where S and N are, respectively, the total number of taxa and the total number of invertebrates. General habitat complexity was assessed by computing a substratum heterogeneity index ($J'_{\text{substratum}}$) and a substratum diversity index ($H'_{\text{substratum}}$), calculated in the same way as species diversity and evenness: $J'_{\text{substratum}} = (H'_{\text{substratum}})/(\text{Maximum } H'_{\text{substratum}})$, where $H'_{\text{substratum}}$ was defined by the frequency of substratum particle classes among the 7 defined classes.

A stream vs. environmental data matrix was constructed and used to perform a Principal Component Analysis after elimination of auto-correlated variables ($R_s \geq 0.8$) and standardization (mean minus each value, divided by the standard deviation) of remaining variables (PRIMER, CLARKE and WARWICK, 2001). This ordination technique was used to detect environmental gradients. The identification of axes' ecological gradients (first and second axes) was made by Pearson correlations between environmental parameters and ordination coordinates. The Kmeans 2 software (LEGENDRE, 1999) was used to perform a non hierarchical classification of sites on the PCA plane defined by the first two axes (ordination co-ordinates were used as site descriptors). The pseudo-F-statistic (CALINSKI-HARABASZ, 1974 in LEGENDRE and LEGENDRE, 1998) was computed in order to evaluate the most suitable number of groups. The objective of this classification was the definition of group of sites with similar environmental characteristics.

Groups defined by ordination and classification were compared with ANOVA in terms of their biological (e.g. H' , S) and environmental (e.g. width, temperature) characteristics. Prior to ANOVA, variables with no normal distribution (KOLMOGOROV-SMIRNOV test) were transformed ($\log(x + 1)$ or $\arcsin$).

To assess the relative contribution of the different groups of environmental variables to benthic composition and community structure, we constructed a stream $\times$ taxa matrix, which was subjected to a similarity analysis (BRAY-CURTIS coefficient, after square root transformation) followed by cluster (average linkage method) and Multidimensional Scaling (MDS) techniques. The stream $\times$ environmental data were also subjected to a similarity analysis (Euclidian distance after data standardization and normalization). The best environmental variables explaining the biological data were assessed by correlating the environmental and taxon similarity matrices with permutation of all variables, according to the BIO-ENV procedure of the PRIMER software (CLARKE and WARWICK, 2001).

3. Results

3.1. General Environmental and Biological Features

Rivers from the North differed from the others in their coarser grain size (Table 2). Whereas rivers from the North and Centre were neutral to acidic (pH 5.4 to 7.3), had low conductivity (21–134 $\mu\text{S cm}^{-1}$) and alkalinity (4–64 $\text{mg l}^{-1} \text{CaCO}_3$), rivers from the South were more alkaline (pH 6.6–9.4; alkalinity 43–471 $\text{mg l}^{-1} \text{CaCO}_3$) and had higher conductivity (160–1151 $\mu\text{S cm}^{-1}$). Current and discharge of all rivers were generally higher in spring than in autumn (Table 2). In autumn, surface flow ceased in two rivers in the South and one river in the North. These streams were therefore reduced to isolated permanent ponds.

A total of 126 taxa were collected from the rivers in the North, 102 from the Centre and 59 from the South. In the North, nearly 70% of individuals were *Leuctra* spp., *Habrophlebia* sp., *Caenis* sp. and *Baetis* spp., Chironomidae and Oligochaeta. In the Centre, nearly 80% of the taxa belonged to the groups: *Ancyclus fluviatilis*, *Leuctra* spp., *Baetis* spp., *Caenis* sp., *Habroleptoides* sp., *Habrophlebia* sp. Chironomidae, Simuliidae and Oligochaeta. In the South, nearly 70% of the individuals belonged to the groups *Caenis* sp., *Choroterpes* sp., *Hydropsyche* spp., Simuliidae and Chironomidae.

The number of taxa per stream was higher in the Centre (40–64) than in the North (31–58) and South (16–28) (Table 3). However, taxon richness [$R_i = (S/\sqrt{N})$], was higher in the North (6 out of 8 samples with $R_i > 1.00$) than in the South (5 out of 8 samples $R_i < 0.50$). In terms of diversity H' , streams in the North and Centre were virtually identical, with values ranging from 1.85 to 2.75, whereas H' in the South ranged from 0.81 to 1.61. Finally, in terms of abundance, streams in the North had lower numbers of individuals than streams in the Centre. Streams in the South were variable with the lowest ($N = 181$) and highest ($N = 13069$) abundances recorded.

3.2. Number of Taxa, Density of Invertebrates and Taxon Richness at Sampling Scale

Numbers of individuals and taxa in all Surber samples were highly correlated, with R_s ranging from 0.61 ($P < 0.001$; $n = 232$, in streams from the North) to 0.77 ($P < 0.001$, $n = 224$ in Southern rivers) (Fig. 2). The rarefied number of taxa (expressed always by $ES(40)$) was significantly correlated with the number of taxa (S) in the samples, with R_s ranging from 0.48 ($P < 0.001$, $n = 177$, in Northern streams) to 0.58 ($P < 0.001$; $n = 177$ for the Centre streams). The number of taxa in each Surber sample ranged from 2 to 23 in the North, 2 to 33 in the Centre and 0 to 14 in the South, whereas the total number of invertebrates in each sample ranged from 5 to 744 in the North, 6 to 1428 in the Centre and 0 to 1679 in the South.

Table 2. Values for physical and chemical parameters in the 12 rivers in spring/autumn (##) 1996. Grain = Median size of substratum particles; AFDM = ash free dry mass of organic content in the substratum. For grain, depth, current and AFDM $n = 118-240$. Other parameters, one spot measurement per season.

Stream	Grain cm	Depth cm	Width m	Current cm s ⁻¹	AFDM g m ⁻²	Discharge m ³ s ⁻¹	Temperature °C	Oxygen mg L ⁻¹	pH	Conductivity µS cm ⁻¹	Alkalinity mg L ⁻¹ CaCO ₃
Olo (N1)	19/18	35/30	9/9	5.1/2.7	2/10	0.17/0.08	11.2/12.0	13.6/13.7	6.6/6.0	21/27	23/23
Pinhão (N2)	12/19	28/22	9/7	7.0/1.5	5/50	0.17/0.02	9.2/10.7	14.0/13.7	6.7/6.0	33/41	44/18
Sordo (N3)	9/12	28/18	7/6	8.9/12.3	10/42	0.17/0.14	8.0/11.4	14.5/13.4	6.5/5.9	40/51	33/23
Tanha (N4)	5/3	39/31	7/7	1.9/0.0	19/11	0.05/0	12.7/13.8	13.7/15.1	6.9/6.5	115/134	50/64
Agadão (C1)	7/6	30/30	7/5	28.0/12.2	6/8	0.56/0.18	12.9/16.3	10.7/10.1	6.7/6.1	28/49	6/4
Laceiras (C2)	6/6	18/14	4/3	21.0/5.6	14/4	0.13/0.03	14.3/19.4	10.3/8.0	6.6/5.4	39/57	4/5
S. João (C3)	7/8	18/18	6/5	35.2/10.4	33/18	0.36/0.10	13.6/14.2	10.8/9.8	7.3/6.1	38/59	11/8
Sóio (C4)	5/5	40/41	9/8	11.1/7.0	7/5	0.39/0.03	14.1/15.7	11.2/9.6	7.1/6.2	27/44	10/5
Ardila (S1)	4/3	30/15	8/8	26.9/0.5	–	0.68/0.01	29.4/19.1	7.0/	8.3/	442/527	156/171
Degebe (S2)	6/6	25/17	10/12	15.0/0	–	0.37/0	28.4/15.1	7.9/8	6.6	533/814	157/210
Vascão (S3)	4/7	15/23	8/14	33.0/0	–	0.40/0	29.6/18.1	7.7/12.8	8.3/8.6	222/1151	471/43
Xévora (S4)	4/4	17/13	13/13	51.0/25.5	–	1.11/0.62	26.2/21.3	11.0/	9.4/	160/202	72/

Geographical coordinates: N1 = 41°22'06"N, 1°14'54"W; N2 = 41°20'39"N, 1°32'36"W; N3 = 41°16'51"N, 1°18'33"W; N4 = 41°13'15"N, 1°24'41"W; C1 = 40°37'15"N, 8°18'05"W; C2 = 40°28'07"N, 8°17'08"W; C3 = 40°05'56"N, 8°14'02"W; C4 = 40°08'13"N, 8°08'41"W; S1 = 39°09'52"N, 7°27'20"W; S2 = 38°17'15"N, 7°33'18"W; S3 = 37°26'57"N, 7°42'04"W; S4 = 39°04'47"N, 7°00'59"W)

Table 3. Biological parameters of the 12 rivers sampled twice a year (spring/autumn, 1996); cumulative values of 25–30 Surber samples.

Rivers	Number of Invertebrates (N)	Number of taxa (S)	Richness $S/\sqrt{N}$	Diversity H'
Olo (N1)	1461/1204	35/40	1.34/1.44	2.18/2.56
Pinhão (N2)	1815/1896	42/46	1.22/1.15	2.55/2.70
Sordo (N3)	1957/1893	58/56	1.26/1.54	2.37/2.55
Tanha (N4)	3820/4367	43/31	0.82/0.51	1.87/1.85
Agadão (C1)	5583/1876	51/56	0.83/1.5	2.05/2.75
Laceiras (C2)	4163/3779	52/62	0.90/1.11	2.41/2.50
S.João (C3)	5228/11482	54/64	0.91/0.71	1.85/2.12
Sótão (C4)	1768/4965	40/51	1.09/0.79	1.91/1.45
Ardila (S1)	2821/1170	22/16	0.45/0.67	0.94/0.81
Degebe (S2)	3090/1489	17/17	0.36/0.57	1.47/1.08
Vascão (S3)	8501/181	21/16	0.28/2.01	1.61/1.51
Xévara (S4)	13069/5287	24/28	0.21/0.48	1.12/1.41

In general, the number of taxa and number of individuals (but not ES(40)) increased with the amount of coarse particulate organic matter in the substratum (*e.g.* in Centre, taxa: $R_s = 0.53$, $P < 0.001$; individuals: $R_s = 0.47$, $P < 0.001$; Fig. 2), and decreased with increasing water depth (*e.g.* in Centre, taxa: $R_s = -0.34$, $P < 0.001$; individuals: $R_s = -0.43$, $P < 0.001$; Fig. 2) and size of the dominant substratum particles (Gmode; Table 4). Depth and organic matter were negatively correlated ($R_s = -0.34$, $P < 0.001$, $N = 177$) suggesting that the amount of organic matter in the substratum may have been influenced by depth. The other measured factors such as biofilm mass did not correlate with any of the biological parameters.

3.3. Diversity at a Geographic Scale

The following environmental parameters were excluded from Principal Component Analysis (PCA) due to high ($R_s > 0.80$) correlations: minimum grain size of substratum particles (correlated with temperature, maximum grain size, mean size of substratum particles, conductivity, alkalinity and pH), substratum heterogeneity J' (correlated with substratum diversity H' and current velocity), substratum diversity H' (correlated with current velocity) and alkalinity (correlated with conductivity). Dissolved oxygen and organic matter were also excluded due to missing values.

The first two axes of the PCA explained 59% of the cumulative variance. The first PCA axis was significantly ($P < 0.005$) correlated with the biological parameters denoting diversity/richness (H' , J' , $S/\sqrt{N}$ and S). The second axis was correlated with the total number of invertebrates. Sites from the North (No), Centre (Ce) and South were segregated, and those from the South formed two groups (SA, SB) (Fig. 3). Rivers in the North, Centre and South had 45, 54, and 17–22 taxa, respectively. Richness in the South being significantly lower than in the other areas ($P < 0.001$; ($N = C$) $\neq$ (SA = SB); Table 5). These regional groups were also statistically different in terms of diversity H' ($P < 0.001$), but not in terms of total number of invertebrates ($P > 0.05$). The four groups also differed in all measured environmental parameters except depth (Table 5).

MDS and cluster analysis of biological data separated streams in the 3 areas in a similar way as PCA did with environmental data. However, one river from the North (N4) and one from the South (S3a) had a distinctly different invertebrate composition (Fig. 4). Only three environmental parameters were highly correlated with the biological data ($R_s = 0.60$; Bio-env analysis): the size of smaller substratum particles, (Gmin), temperature, and pH.

Figure 2. Relationships between (a) the number of taxa and total invertebrates; (b) total number of invertebrates and organic matter content in the substratum; and (c) number of taxa and depth. Only shown streams from Central Portugal.

Table 4. Number of significant correlations (Spearman rank coefficient) between the biological parameters number of taxa (S), number of taxa for a standard sample of 40 individuals (ES(40)), total number of individuals (N), and several parameters measured in the exact location where Surber samples were taken. Gmode = size of the more abundant substratum particles in a sample; Gmin = smallest size class; Gmax = largest size class; + = positive correlation; - = negative correlation. Superscripts show the number of comparisons (*e.g.* 3 areas $\times$ 4 streams $\times$ 2 seasons = 24).

	S	N	ES(40)
Organic Matter ¹⁶	7(+)	8(+)	1(-)
Gmode ²⁴	4(-)	6(-)	3(-)
Depth ²⁴	5(-), 1(+)	2(-), 1(+)	2(-)
Current ²⁴	4(+), 1(-)	3(+)	1(-)
Gmax ¹⁶	2(-), 1(+)	3(-)	1(-)
Gmin ¹⁶	3(-), 1(+)	2(-), 1(+)	1(-)

Figure 3. Principal Component Analysis of environmental data of streams located North (N), Centre (C) and South sampled in spring (SB) and autumn (SA).

4. Discussion

We analysed diversity/taxa richness at two scales: (a) a small scale – sample unit, and (b) a large scale – climatic and geomorphologic gradient. Our first prediction was that at a small scale (Surber sampling unit or micro-habitat), the number of taxa would increase with the increase in substratum heterogeneity and the availability of organic matter.

The first prediction was not fully confirmed. Surber samples taken from structurally more complex habitats, defined by the size of dominant substratum particles and the number of

Table 5. Comparison of several biological and environmental parameters among the 4 groups obtained from Principal Component Analysis and Cluster Analysis (Ce = Centre, No = North, SA and SB = two groups from South). Parametric (F value) and Non-parametric (H value) ANOVAs followed by Tukey HSD and Dunn's range tests, respectively. Lines connect groups that are not significantly different.

Parameter	Statistics	Tukey/Dunn's tests
Number of taxa	F = 30.774, P < 0.001	<u>Ce > No > SB > SA</u>
H'	F = 11.987, P < 0.001	<u>Ce > No > SB > SA</u>
Total number of invertebrates	F = 2.758; P > 0.05	
Temperature	H = 18.803, P < 0.001	<u>SB > SA > Ce > No</u>
Dissolved oxygen	F = 22.685, P < 0.001	<u>No > SA > Ce > SB</u>
pH	F = 12.455, P < 0.001	<u>SB > SA > Ce > No</u>
Alkalinity	H = 19.182, P < 0.001	<u>SA > SB > No > Ce</u>
Conductivity	H = 15.968, P = 0.001	<u>SA > SB > Ce > No</u>
O. M. in the water column	H = 16.378, P < 0.001	<u>Ce > SA > SB > No</u>
Depth	F = 2.051, P > 0.05	
Width	H = 12.431, P < 0.01	<u>SA > SB > No > Ce</u>
Discharge	F = 8.929, P < 0.001	<u>SB > Ce > SA > No</u>
Current velocity	F = 5.125, P < 0.01	<u>SB > Ce > SA > No</u>
Substratum J'	F = 19.786, P < 0.01	<u>SB > Ce > SA > No</u>

substratum size classes, did not contain more taxa or individuals. This could be for three reasons. Firstly, it is plausible that our measurements of substratum heterogeneity were unsatisfactory. It is very difficult to express the complex substratum mixtures in a simple index, although several qualitative scores have been suggested (*e.g.* embeddedness, BARBOUR *et al.*, 1999). Our measurements of heterogeneity therefore may not be relevant to macroinvertebrates. Secondly, although other authors have reported lower numbers of individuals and taxa in finer substrata (*e.g.* DEMARCH, 1976; GRAÇA *et al.*, 1994), in the present study substratum particles were in general >10 mm, which means that the very fine, homogeneous substrata (sand) referred to by other authors were not present in our study. The third reason for the discrepancy may be an overriding effect of other factors.

Correlation analyses showed that the other factors affecting the number of taxa and total number of invertebrates included the organic matter content of sediments and depth. Organic matter and depth were indeed negatively correlated, which is consistent with previous reports that retention of leaves decreased with depth in rivers (*e.g.* CANHOTO and GRAÇA 1998): leaves tend to accumulate in shallow areas, near the margins or in areas where retentive structures (such as twigs) project from the substratum to the surface.

Figure 4. Multidimensional scaling (top) and cluster analysis (bottom) of sites, based on taxonomic composition. Dashed lines denote groups of sites defined by the PCA in Figure 3.

The increase in invertebrate numbers and taxa with an increase in detritus biomass in sediments is consistent with our second prediction. Since detrital organic matter is the main energy source for invertebrates inhabiting low order forested streams (WALLACE and WEBSTER, 1996) and it usually has a patchy distribution, one would expect to find more taxa in areas where leaves accumulate. However, invertebrates may colonize patches with particulate organic matter because of the availability of food (PIDGEON and CAIRNS, 1981), or, alternatively, because factors influencing organic matter distribution also influence invertebrates. Several authors have indeed confirmed the prominent role of detritus in the distribution, and density of invertebrates. ARUNACHALAM *et al.*, (1991) reported higher densities of invertebrates in leaf packs and in shallow areas of a river. In a manipulative experiment with baskets containing sediments differing in heterogeneity and detritus content, WILLIAMS, (1980) showed that higher biomass and numbers of invertebrates were attained in treatments with more detritus, independently of substratum heterogeneity. In another manipulative experiment, CULP *et al.*, (1983) and CULP and DAVIES, (1985) reported that the addition of organic material to inorganic sediments of several particle sizes generally resulted in an increase in the number of individuals and/or in total invertebrate biomass. Detritus accumulation may enhance habitat heterogeneity, but several manipulative experiments have shown that the food value of coarse particulate organic matter, and not heterogeneity, is a main

determinant of preferential colonization of substrate by invertebrates (RICHARDSON, 1992; GRAÇA and PEREIRA, 1995). In another study, CLARKE and SCRUTON, (1997) examined the relationship between chemical and physical parameters of 20 streams and the diversity and density of macroinvertebrates and found a significant relationship between invertebrate biomass and organic matter mass and temperature in the studied streams.

Therefore, we propose the following mechanism to explain patterns of taxa richness at small scales in streams. Current assorts the inorganic particles of substratum in the river bed. Current also transports particulate organic matter and invertebrates. Some studies have shown that colonisation of new substrata occurs mainly by drift and not by active movement within the substratum (*e.g.* MATTHEI *et al.*, 1997). Drifting invertebrates are likely to remain in a patch if organic matter is present. As more invertebrates accumulate in patches with coarse particulate organic matter, the chance of having more taxa therefore also increases. In our study, when the number of taxa was standardized to a constant number of individuals, the relationship of organic matter *vs* number of taxa disappeared. This shows that the increase in number of taxa is indeed a consequence of more invertebrates in a patch.

In a similar study, MIYAKE and NAKANO, (2002) also found that at patch scale, the strongest determinant of taxa richness was particulate organic matter, overwhelming the importance of substratum stability and periphyton biomass. Furthermore, they found that the greater taxon richness in a patch was a consequence of the accumulation of more macroinvertebrates as in our study.

A final prediction of our study was that, at a large scale, Mediterranean streams would have reduced number of taxa due to their extreme environmental variability (MALTCHIK *et al.*, 1996). This was indeed the case. Streams from the North and Centre of Portugal were not substantially different in terms of climatic characteristics and water chemistry and had significantly higher diversity than rivers in the South. Our results confirm those previous studies in which rivers in the North and South of Portugal were studied intensively (*e.g.* CORTES, 1992; MORAIS, 1995).

Unlike the North and Centre, rainfall is scarcer and less predictable in the South. Moreover, due to the relatively flat landscape, most streams and rivers are reduced to ponds with no surface flow during some months of the year. With no flow, the conditions are lentic. The presence of decaying macrophytes and high temperatures (several days in the year with air temperatures typically around 40 °C) cause high microbial activity and decreased dissolved oxygen concentrations. Comparatively fewer organisms are capable of colonizing these habitats, especially if the streams dry out, or if summer lentic conditions shift abruptly to lotic conditions with the first rains.

There is a large amount of literature on the relationship between environmental parameters and the faunal composition of streams. In some geographical areas it is possible to predict macroinvertebrate fauna composition from a few basic environmental measurements (*e.g.* WRIGHT *et al.*, 1998). However, the relationship between diversity/richness/biomass and environmental conditions is less clear. For example, CLARKE and SCRUTON, (1997) reported no significant correlation between numbers, biomass and diversity of macroinvertebrates and several environmental parameters including pH, temperature, mean depth, velocity, conductivity and nitrate concentration in 20 streams in Newfoundland, Canada. In contrast, TOWNSEND *et al.*, (1983) found that, among a set of 34 stony riffle sites, acid sites had lower numbers of individuals and low species richness (range pH 4.8–6.5), and CLENAGHAN *et al.*, (1998) reported that taxa richness, density of invertebrates and diversity increased along a river continuum with increases in pH, hardness and nutrients.

In our case, the streams with lower pH, conductivity and alkalinity (North and Centre) had the higher species diversity, but the range of pH and alkalinity values in our streams was higher than reported in the studies cited above. We believe that climatic and geomorphologic factors overrode chemical characteristics of the water and maybe other physical factors, in our study. In terms of applicability of our data (*e.g.* Water Framework Direc-

tive), a question arises: for a monitoring program, should Mediterranean systems be sampled with the same frequency considering their stochastic nature? On the other hand, the definition of predictive models for benthic communities on the basis of the environmental parameters demands high effort and associated costs, since this work showed the difficulty in establishing cause-effect relations for such a diverse spatial ecological gradients from North to South.

5. Acknowledgements

This study was financed by the Fundação para a Ciência e Tecnologia, through the project PRAXIS/2/2.1/BIA/113/94. Thanks ALBERTO BASSET, ERIC PATTÉE, VLADISLAV GULIS for comments and corrections to an earlier version of the manuscript.

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Manuscript received August 18th, 2003; revised December 15th, 2003; accepted December 18th, 2003