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LEAF BREAKDOWN RATES: A MEASURE OF WATER QUALITY?

Key words: lotic systems, organic pollution, leaf breakdown, macroinvertebrates.

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Abstract

The breakdown rates of *Alnus glutinosa* leaves and the structure of macroinvertebrate communities were used to evaluate the impact of the village of Montalegre (Portugal) on the water quality of the Cávado river. Chemical and microbial analyses of stream water indicated a high organic load in the vicinity of the village. The abundance of macroinvertebrates associated with leaves increased along the pollution gradient, whereas richness of the community decreased. Biotic indices and multivariate analysis applied to aquatic macroinvertebrate communities discriminated polluted from non-polluted sites. Exponential breakdown rates of alder leaves were high (0.014 to 0.060 day^{-1}) and the differences observed among sites suggested that nutrient enrichment stimulated leaf breakdown significantly. Leaf breakdown rates have not reflected improved biotic conditions as assessed by biotic indices at the most downstream site. These results suggest that both data from the structure and function of a stream are important for assessing water quality.

1. Introduction

A broad range of changes on environmental conditions imposed by man can promote stress on freshwater ecosystems, which is expected to influence the structure and function of benthic communities as well as processes such as leaf breakdown. Several factors are known to affect leaf breakdown rates. These include temperature (e.g., ROWE *et al.*, 1996), stream water chemistry (e.g., SUBERKROPP and CHAUVET, 1995), leaf species (e.g., IMBERT and POZO, 1989) and extent of colonization by macroinvertebrates and microorganisms (for a review see BOULTON and BOON, 1991; GRAÇA, 1993; WEBSTER *et al.*, 1995). Since leaf breakdown rates are sensitive to changes in the environment and are easy to measure (GRAÇA, 1993), they could be potential tools to assess water quality. The data available in the literature concerning the effect of pollution on leaf breakdown are contradictory (RAVIRAJA *et al.*, 1998). Faster leaf breakdown is commonly observed in streams enriched with nutrients (e.g., MEYER and JOHNSON, 1983). However, this enrichment is often accompanied by other pollutants that may have the opposite effect and cause slower breakdown (WEBSTER *et al.*, 1995).

The aim of this research was to investigate whether leaf breakdown rates can be used to evaluate the impact of organic pollution on water quality in a stream. For this purpose, physicochemical and microbiological parameters were determined along a longitudinal pollution gradient of Cávado river, and related to breakdown rates of *A. glutinosa* leaves and macroinvertebrate communities.

2. Materials and Methods

2.1. Study area and sampling sites

Cávado river runs from Serra do Larouco (North of Portugal, 1400 m altitude) to the Atlantic Ocean over a distance of 130 km. It flows over granite rocks from hercinic age with some metasediments from the Paleozoic age in both the most upstream and downstream section. Heavy rainfalls between December and April, with short periods of ice and snow, characterize the climate during winter, while the arid period is from June to September.

The study stretch extended from the source of Cávado river, through nearly 16 km (Figure 1), with the altitude ranging from 1200 to 900 m. Agriculture and extensive cattle breeding are the main human activities in the catchment. Table 1 shows some physical characteristics of the sampling stations. Two sites (L1 and L2) were located above the village of Montalegre and its wastewater treatment plant, L3 and L4 were located near the village, and three sites (L5, L6 and L7) were located below (Figure 1).

2.2. Physicochemical and microbiological analyses

Water temperature and pH were measured *in situ* at each sampling point with field probes. Measurements were performed weekly during September 1994. For further chemical and microbiological analyses, water samples were collected during the first and last weeks from the following sampling sites: L2, L3, L4, L5, L6 and L7. Samples were taken in sterile glass bottles, transported in a cold box (4°C) and analysed within 24 h. Chemical oxygen demand (COD) was measured using a kit (nº 250323, mod. C2/25, WTW) and a digester (CSB-COD-CR 1100, WTW) according to METCALF and EDDY (1991). A HACH DR/2000 photometer was used for the analysis of total iron (TPTZ reagent, HACH), orthophosphate (molybdate reagent, HACH) and nitrate (Nitra-Ver 5 reagent, HACH). Ammonium (kit nº250323, mod. A5/25, WTW), nitrite (kit nº250385, mod. N4/20, WTW) and sulphate (kit nº250414, mod. 14548, WTW) were quantified with a spectrophotometer (MPM 1500, WTW). The colony-forming units (CFUs) of total heterotrophs, and the most probable number (MPN) of total and faecal coliforms as well as *Streptococcus faecalis* were quantified according to Standard Methods for the Examination of

Water and Wastewater (AMERICAN PUBLIC HEALTH ASSOCIATION, 1989).

2.3. Macroinvertebrate sampling and leaf mass loss

Macroinvertebrates were harvested in September 1994 either with leaf bags or with a hand net (60 x 30 cm; 0.6 mm mesh size). Hand net sampling was performed by both kicking and sweeping all available biotopes in proportion to their occurrence during 5 min per station (L2, L3, L4, L5 and L7). Additionally, both stones and submerged vegetation were examined to collect the benthic macroinvertebrates, which were subsequently preserved in 4% formaldehyde (pH 7.0). Leaves of *A. glutinosa* were collected just before abscission, stored air dried and then oven dried at 60 °C for 48 h just before use. The leaves were weighed into 8 g groups and placed in plastic mesh bags (volume 1248 cm^3, mesh size 10 mm). The bags were sealed and anchored to stones in the river at five of the sampling stations (L1, L2, L3, L4 and L7). A total of twelve bags were placed in the stream at each sampling station. Three leaf bags were retrieved from each site weekly over a period of one month. To determine the leaf mass loss, the leaves were rinsed with water, dried at 60 °C to a constant mass (48 ± 24 h) and weighed to

the nearest 0.01 g. The macroinvertebrates were sorted and preserved in ethanol (70%, v/v). Macroinvertebrates, collected from leaf bags and by hand net, were enumerated and identified to genus, when possible. *Taxa* were assigned to functional feeding groups according to MERRITT and CUMMINS (1996).

2.4. Data analysis

The macroinvertebrate communities were analysed in terms of total abundance, richness (MARGALEF, 1958) and biotic indices, namely Belgian Biotic Index (BBI- DE PAUW and VANHOOREN, 1983) and Biological Monitoring Working Party, adapted to the Iberian Peninsula (BMWP´- ARMITAGE *et al.*, 1983; ALBA TERCEDOR and SANCHEZ-ORTEGA, 1988; RICO *et al.*, 1992).

To analyse the distribution of macroinvertebrates sampled with leaf bags, Factorial Correspondence Analysis (FCA) was used (VOLLE, 1993). The analysis was based on average value from replicate leaf bags collected after three weeks of immersion. Dissimilarities in faunistic profiles among sampling sites were

quantified by the Bray-Curtis coefficient (FAITH *et al.*, 1987), after $\log_{10}(x+1)$ transformation. The resulting symmetric matrices were subjected to cluster analysis using the Unweighed Pairgroup Method Average (UPGMA - HELLAWELL, 1978).

The abundance of macroinvertebrates assigned to different functional feeding groups was compared among sampling sites by analysis of variance (ANOVA), after $\log_{10}(x+1)$ transformation. Additionally, Tukey´s HSD (Honestly Significant Difference) test was carried out to determine in which sites significant differences occurred (ZAR, 1996).

Leaf breakdown rates were obtained by fitting the percentage of dry weight loss to the exponential model $W_t=W_o \cdot e^{-kt}$ (PETERSEN and CUMMINS, 1974), where k is the exponential breakdown coefficient, W_t is the dry weight of leaves remaining after time t from the initial amount W_o. Regression lines (ln transformed data) were compared by analysis of covariance followed by Tukey´s HSD test (ZAR, 1996).

3. Results

3.1. Physicochemical and microbial parameters

Mean values for temperature and pH varied little either among the sampling stations or the sampling time as follows: 12.5 ± 0.5 °C and 6.4 ± 0.2, respectively.

The presence of the village of Montalegre and its wastewater treatment plant led to an increase in COD, ammonium, phosphate and nitrate concentration (Figure 2) in Cávado river. The highest values for COD (Figure 2A), phosphate and ammonium (Figure 2B) were found at L4, while the lowest ones were obtained at L2, indicating a high organic load associated with the effluent discharge. The highest concentration of nitrate (Figure 2B) occurred at L5, probably as a consequence of the ammonium oxidation between sites L4 and L5. The values obtained for the level of nitrite, sulphate and total iron were similar among sampling sites (Figure 2B). The input of sewage effluent resulted in a 40-fold increase in the abundance of heterotrophic microbial populations from L2 to L4 with a decrease of similar magnitude at more downstream sites (Figure 3A). A similar pattern was observed for total and faecal coliforms as well as for *Streptococcus faecalis* (Figure 3B), with a

maximum of 160 MPN per ml at L4. These results were in agreement with the chemical data presented above, which revealed high organic load associated with the village and its wastewater treatment plant.

3.2. Structure, sensitivity and function of the macroinvertebrate community

Leaf bag studies carried out along the sampling stations showed that macroinvertebrate abundance and richness increased with exposure time, reached maxima after two or three weeks and subsequently decreased (not shown). Therefore, samples collected after three weeks of immersion were used in the present study. The macroinvertebrate data obtained with both sampling methods (leaf bags and hand net) showed the highest values for abundance (Figure 4A) and the lowest for *taxa* richness (Figure 4B) immediately below the effluent discharge (L4). According to the BBI (DE PAUW and VANHOOREN, 1983) applied to hand net samples (Figure 4C), the water was slightly polluted at L3 and L4, while the other sampling stations were classified as non-polluted (L2, L5 and L7). In addition, the same index applied to the macroinvertebrate assemblages colonizing leaf bags (Figure 4C) indicated moderate pollution at L3

and L4, whereas the other sites (L1, L2 and L7) were classified as non-polluted. Regarding BMWP´ (Figure 4D), the lowest value was observed at L4 with both sampling methods, allowing the classification of this site as seriously polluted, according to the quality classes proposed by RICO *et al.* (1992). On the other hand, L1, L2 and L7 were classified as clean. L3 was classified either as having polluted water or water with some disturbance, depending on the hand net or leaf bag sampling methods, respectively. L5 was classified as having water with some disturbance, which could be an indicator of recovery below L4.

The distribution of the macroinvertebrate assemblages colonizing leaf bags by FCA, along the sampling sites, revealed that the first factorial plane explained 80% of the total inertia, 51.9% was explained by the first factor (horizontally) and 29.1% by the second one (vertically). Factor 1 separated L3 and L4 from L1, L2 and L7 and factor 2 distinguished L1 and L2 from L7, L3 and L4 (Figure 5A). These results suggested the division in 3 groups: L1 and L2, L3 and L4, and L7. The analysis of relative and absolute contributions (not shown) suggested that genus *Erpobdella*, was strongly associated with L3 and L4, whereas genera *Leuctra*, *Protonemura* and *Chaetopteryx* were related with L1 and L2. On the other hand,

genera *Perla*, *Sericostoma* and *Calopteryx*, and Tipulidae were closely associated with L7. The taxonomic similarity among the sampling stations, classified by UPGMA cluster analysis (Figure 5B), showed high faunistic similarity between L1 and L2 and between L3 and L4. Sampling site L7 was closer to the first group than to the second one. This hierarchical classification corroborated the results from FCA (Figure 5A).

Differences among sampling sites in terms of functional feeding groups of macroinvertebrates associated with leaf bags were established considering L1 and L2 as reference upstream sites, since data from biotic indices proved conditions of clean water and they exhibited high faunistic similarity. Results shown in Figure 6 indicated that significant differences were found (ANOVA, $p < 0.001$) for collector-gatherers, scrapers and shredders, but not for predators and collector-filterers. Numbers of shredders per leaf bag were high at reference upstream sites, fell abruptly at L3 and L4, and increased further downstream. Tukey´s test revealed that the number of shredders at L3 and L4 was significantly lower than at all other sites ($p < 0.001$), whereas no significant differences were found between either the upstream sites (L1 and L2) and L7 ($p = 0.840$) or L3 and L4 ($p = 0.911$). The absence of shredders at the polluted sites

(L3 and L4) was expected since this group includes *taxa* sensitive to pollution such as Plecoptera and some Trichoptera (Figure 5A). Numbers of collector-gatherers were similar at all sampling stations, with the exception of L7, where significantly higher numbers were found ($p < 0.001$). Although average scraper densities were highest at L4, no significant differences could be detected in this functional group between this site and L3 ($p = 0.430$).

3.3. Leaf breakdown rates

Exponential breakdown rates of alder leaves were high (Petersen and Cummins, 1974) and ranged from 0.014 to 0.060 per day (Table 2). Analysis of covariance revealed significant differences ($F = 14.105$ and $F = 2.056$; $p < 0.05$) in the leaf breakdown rates among the sampling stations. Leaf breakdown was significantly faster at polluted sites (L3 and L4) compared with upstream sites (L1 and L2). No significant differences ($q < 3.97$; $p > 0.05$) were found among L3, L4 and L7.

4. Discussion

In the present study, the water quality assessed as COD (Figure 2A) and numbers of total heterotrophic microorganisms (Figure 3A) showed clear evidence of a point source of organic pollution in the Cávado river near the village of Montalegre (sampling stations L3 and L4). In this environment, the macroinvertebrate community (sampled with both a hand net and leaf bags) responded with a decrease in *taxa* richness (Figure 4B) and an increase in abundance (Figure 4A) of more tolerant organisms (Figure 5A). Although changes in richness have been used as indicators of environmental stress, these measures do not take into account the tolerance of individual *taxa* to pollution (WASHINGTON, 1984). Biotic indices such as BBI and BMWP´ can overcome this limitation. Analysis of these indices indicated that L3 and L4 were polluted, while the other sites were classified as non-polluted (Figure 4C and D). However, the degree of pollution evaluated by each index was different, depending on either the sampling methods or the biotic index used. BMWP´ detected differences in water quality between L3 and L4 sampling sites, which seems to be in accordance with the chemical (Figure 2) and microbial analyses (Figure 3). These results suggested that both indices were effective indicators of water quality

in Cávado river, although BMWP´ seemed to be more sensitive to detect changes induced by organic pollution. In spite of this, both biotic indices were not sensitive to the relatively high level of ammonium and nitrate found at the most downstream site.

Changes in water quality have also been evaluated by alterations in the structure of macroinvertebrate communities as measured by multivariate analyses (e.g., GUINAND *et al.*, 1996). FCA and the UPGMA cluster analysis of macroinvertebrates, associated with leaf bags, discriminated polluted sites (L3 and L4) from all others, with L7 being closer to the upstream (L1 and L2) than to the polluted ones (Figure 5). Differences in terms of faunistic similarity between upstream sites and L7 could be related to the shift in macroinvertebrate community structure as a consequence of differences in physical factors such as an increase of width and stream order (Table1) and/or an increase of nitrate and ammonium content (Figure 2B).

It has been proposed that leaf breakdown rates could be used as a tool to evaluate the impact of anthropogenic disturbances in lotic systems (WEBSTER and BENFIELD, 1986). There is considerable evidence that shredders can play a significant role in leaf breakdown (e.g. GESSNER *et al.*, 1991). In the present study, the decline of

shredders at polluted sites did not result in a decrease in leaf exponential breakdown rates. On the contrary, significantly higher breakdown rates were found at polluted sites (L3 and L4) compared with the upstream sites (L1 and L2). The high breakdown rates at polluted sites (Table 2) may have been due to increased concentration of nutrients (Figure 2), which could have stimulated microbial decomposing activity. These results are in accordance with increased leaf breakdown rates in nutrient-rich streams found by other authors (MEYER and JOHNSON, 1983; SUBERKROPP and CHAUVET, 1995). However, no significant differences were found in the breakdown rates between the polluted sites (L3 and L4) and L7, the most downstream site located 8.5 km below the village, classified as non-polluted by biotic indices. The rapid decomposition at L7 was attributable to the dense colonization by macroinvertebrates, especially shredders (Figure 6), and microbial decomposing activity supported by relatively high levels of nitrate and ammonium (Figure 2A).

In summary, the structure of macroinvertebrate communities associated with decomposing alder leaves responded to changes in chemical and microbial parameters induced by the presence of Montalegre village and its wastewater treatment plant. Shredders

were clearly not responsible for the significantly faster decomposition at the most organically polluted sites. Leaf breakdown rates were significantly stimulated with increased concentration of nutrients, mainly ammonium and nitrate, but have not reflected improved biotic conditions as assessed by biotic indices at the most downstream site. Overall, the results of this work suggested that data from both the structure of macroinvertebrate communities and leaf breakdown rates should be used for assessing water quality.

5. Acknowledgements

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LEGENDS

Figure 1. Location of sampling sites along the upper course of Cávado river.

Figure 2. Chemical parameters quantified along sampling sites. A: Chemical oxygen demand (COD) and B: Concentration of ions. Symbols: A, nitrite; ●, sulphate; C, total iron; E, phosphate; H, ammonium; and Ñ, nitrate. Arrows indicate the discharge of the effluent. Analyses were not performed on samples from L1.

Figure 3. Quantification of microbial populations. A: Total heterotrophs and B: Total coliforms (E); faecal coliforms (Ñ); and *Streptococcus faecalis* (A). Arrows indicate the discharge of the effluent. Analyses were not performed on samples from L1.

Figure 4. Measures of diversity and biotic indices of the macroinvertebrate community sampled by hand net (open symbols) and with leaf bags (closed symbols). A: Abundance (number of individuals), B: Margalef richness index, C: BBI, and D: BMWP´. Arrows indicate the discharge of the effluent.

Figure 5. Multivariate analysis. A: FCA ordination (factorial plane 1/2) of the macroinvertebrate *taxa* colonizing the leaf bags after 3 weeks in

the stream as a function of sampling sites. *Taxa* with the highest relative and absolute contributions are shown in bold. B: UPGMA cluster analysis of the taxonomic similarity of macroinvertebrate communities among the sampling sites as based on Bray-Curtis distances.

Figure 6. Distribution pattern of functional feeding groups colonizing the leaf bags after 3 weeks of immersion along the sampling sites of Cávado river. Error bars indicate ±1 SD of three replicate measurements.

Table 1. Characterization of the sampling sites along the Cávado river.

Sampling site	Distance from source (km)	Width (m)	Riparian vegetation	Substratum type	Stream order
L1	4	1.5	present[#]	rock, gravel, sand, vegetation	2
L2	5.5	2	present[#]	pebbles, gravel, sand, vegetation	2
L3	10	3	absent	gravel, mud, algae	2
L4	11	3.5	present[#]	mud, algae	2
L5	14	5	absent	gravel, sand, vegetation	2
L6	17	6	absent	gravel, sand, vegetation	2
L7	20	7	present[#]	pebbles, gravel, vegetation	3

Mean water depth was 40 cm at all sampling sites.

[#] Composed mainly of *Salix* spp., *Quercus pyrenaica*, *Quercus robur* and *A. glutinosa*.

Table 2. Exponential breakdown rates (k) of *A. glutinosa* leaves along the sampling sites.

Sampling site	k ± SE (day^{-1})	r^2	N
L1	0.014 ± 0.013	0.58	15
L2	0.024 ± 0.016	0.74	15
L3	0.046 ± 0.017	0.95	9
L4	0.047 ± 0.011	0.94	9
L7	0.060 ± 0.013	0.88	15

N, number of samples; r^2, coefficient of determination; and SE, standard error.

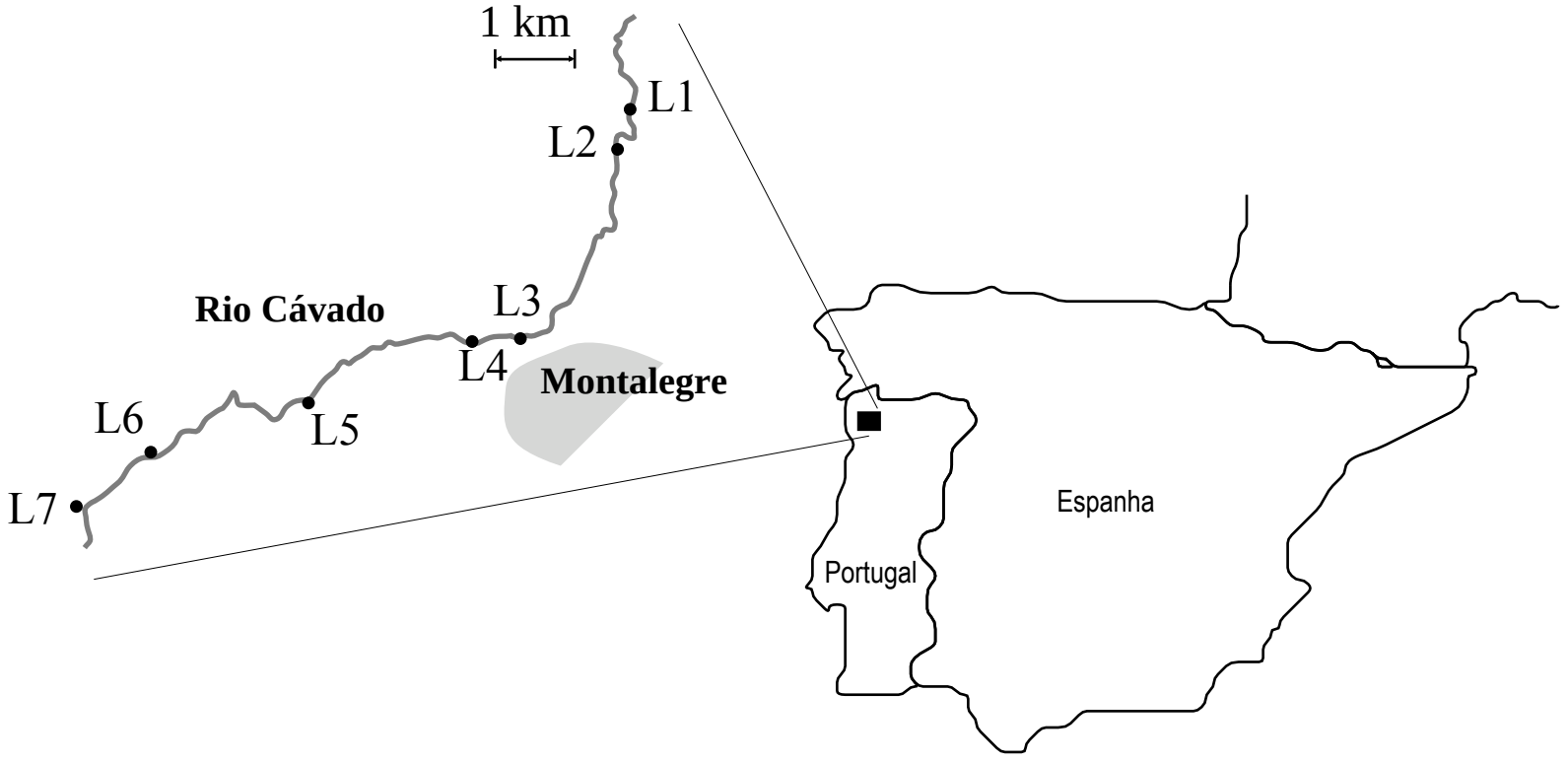
1 km
L1
L2
L3
L4
L5
L6
L7
Rio Cávado
Montalegre
Espanha
Portugal

Fig.1

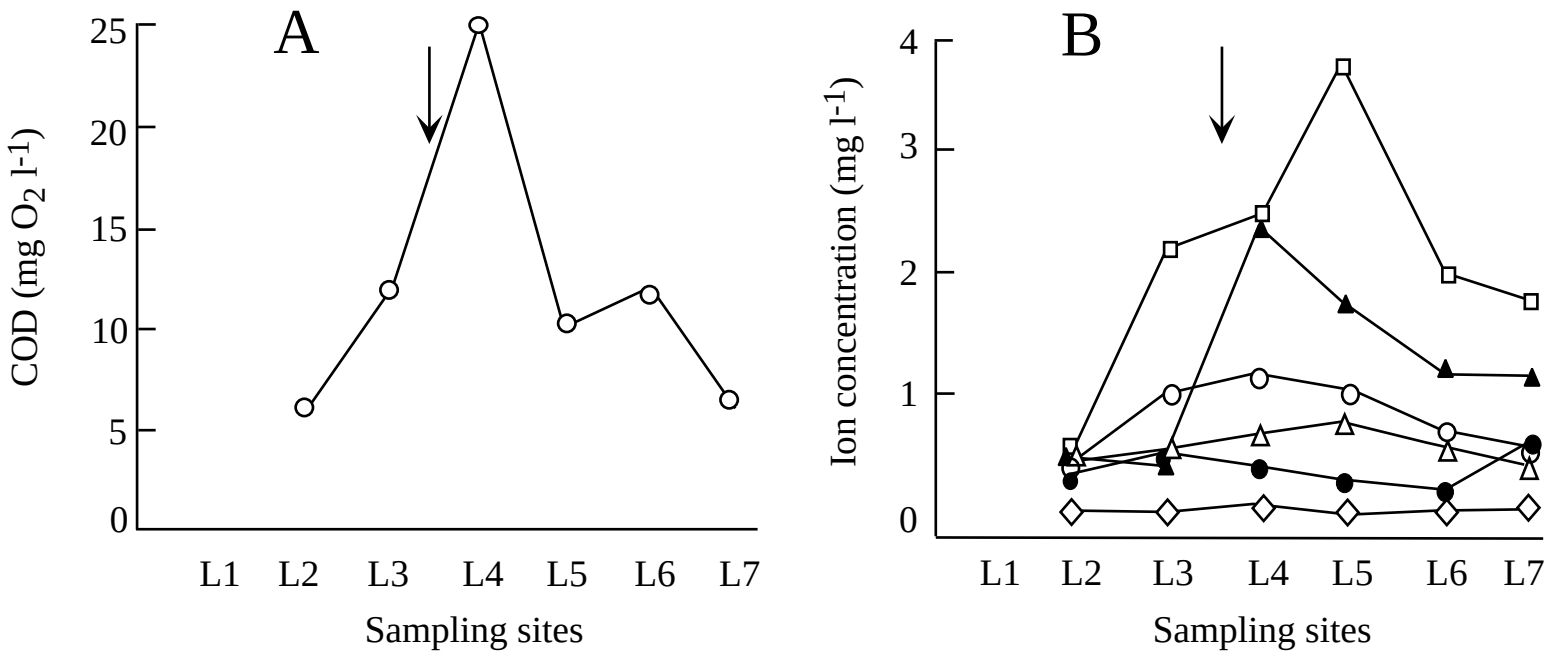
A
B
COD (mg O_2 l^{-1})
Ion concentration (mg l^{-1})
0
5
10
15
20
25
0
1
2
3
4
L1
L2
L3
L4
L5
L6
L7
Sampling sites
Sampling sites

Fig.2

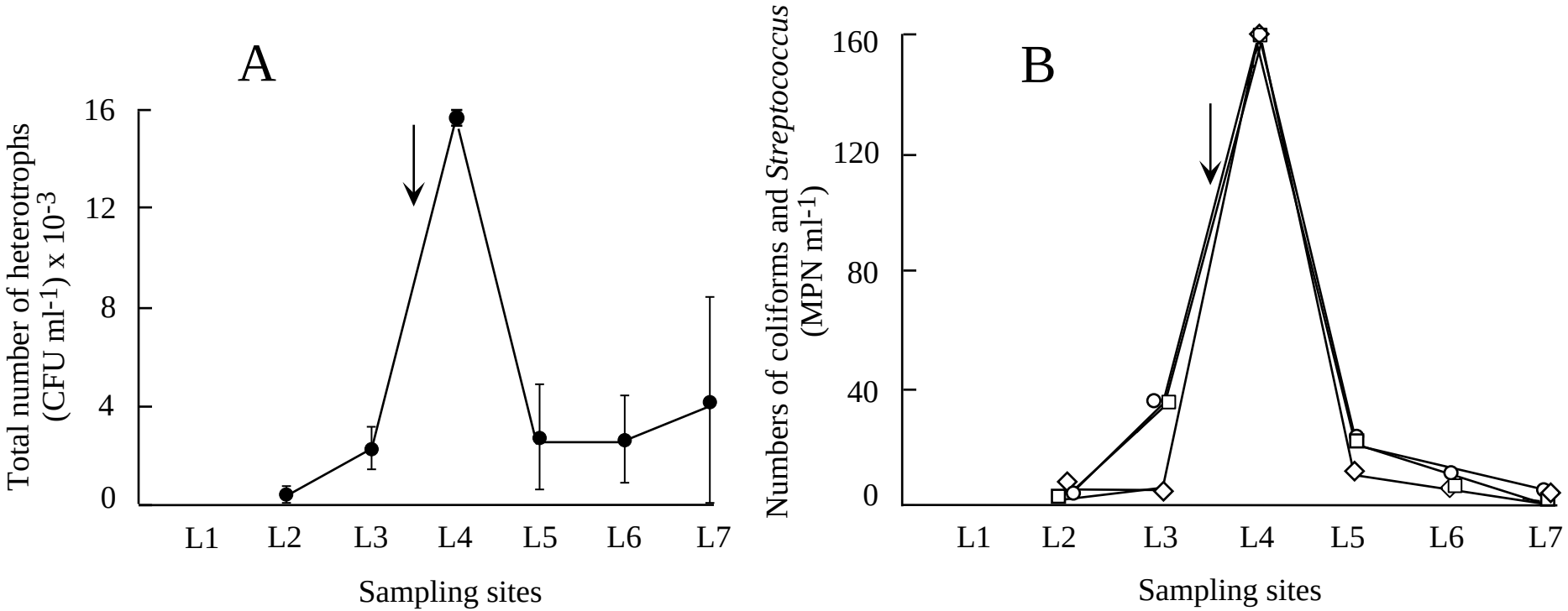
A
Total number of heterotrophs
(CFU ml-1) x 10-3
16
12
8
4
0
L1
L2
L3
L4
L5
L6
L7
Sampling sites
B
Numbers of coliforms and Streptococcus
(MPN ml-1)
160
120
80
40
0
L1
L2
L3
L4
L5
L6
L7
Sampling sites

Fig.3

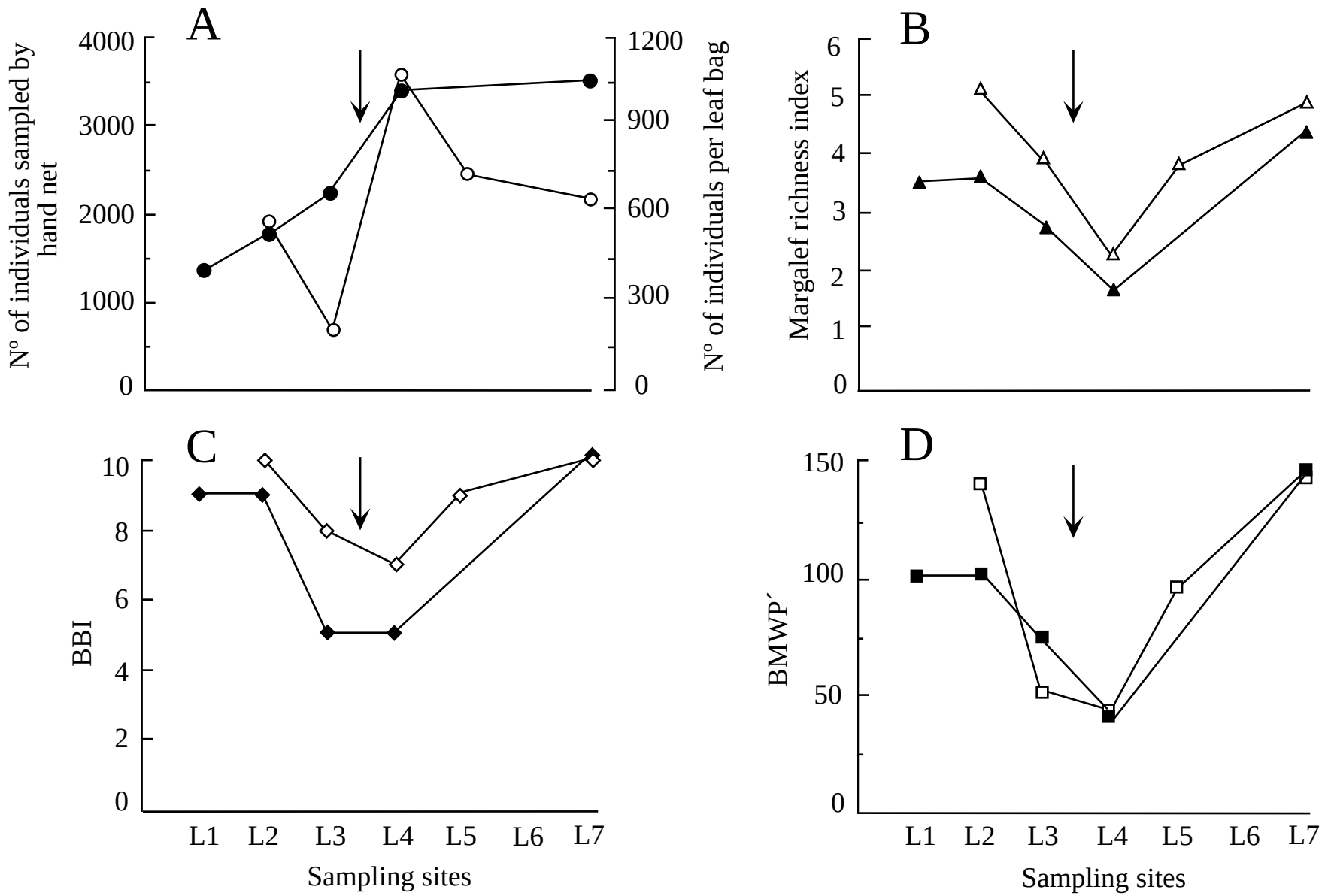
A
Nº of individuals sampled by hand net
Nº of individuals per leaf bag
4000
3000
2000
1000
0
1200
900
600
300
0
B
Margalef richness index
6
5
4
3
2
1
0
C
BBI
10
8
6
4
2
0
D
BMWP´
150
100
50
0
L1
L2
L3
L4
L5
L6
L7
Sampling sites

Fig.4

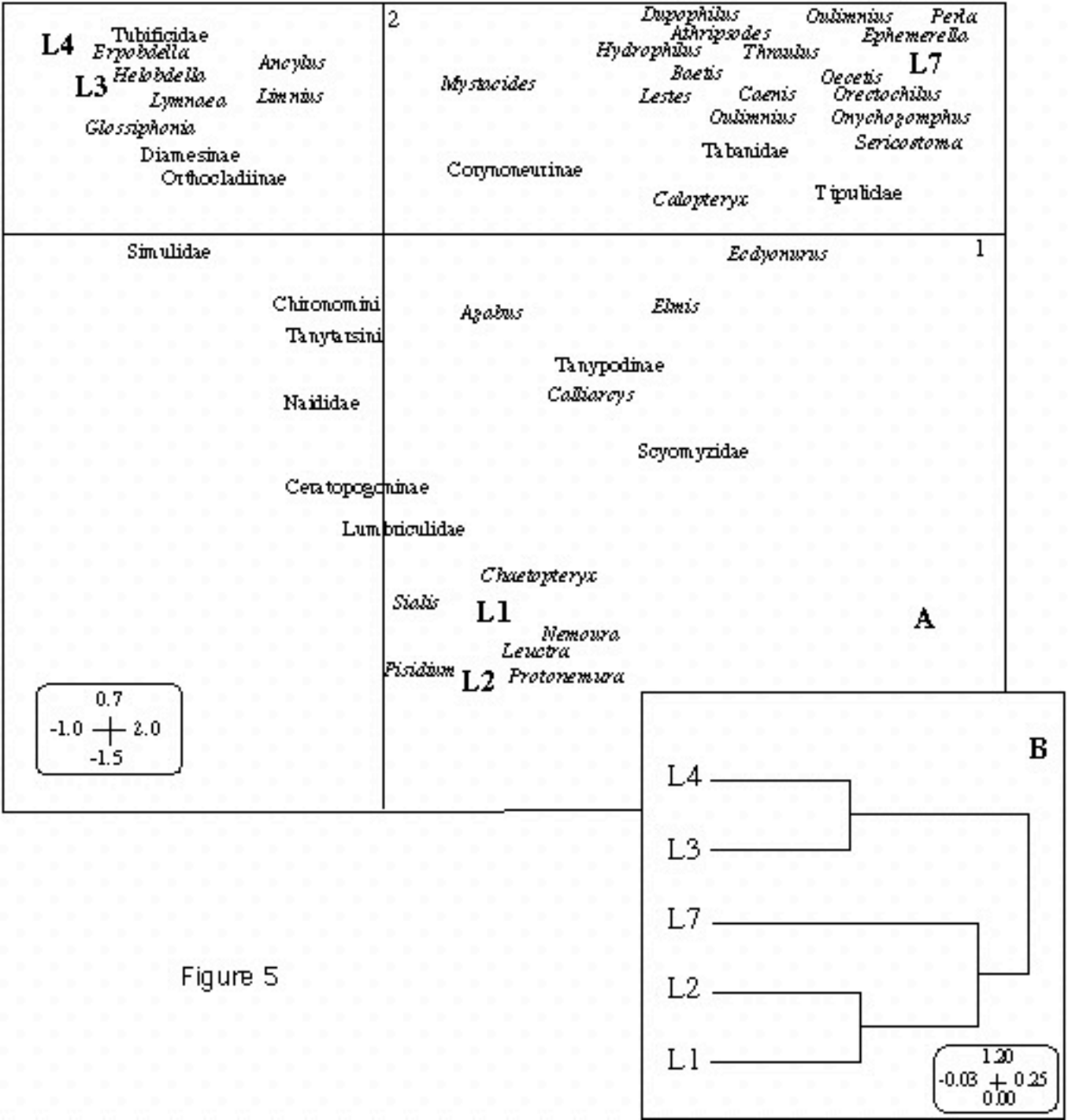
L4
Tubificidae
Erpobdella
Helobdella
L3
Lymnaea
Glossiphonia
Diamesinae
Orthocladiinae
Ancylus
Limnius
2
Dupophilus
Oulimnius
Perla
Athripsodes
Ephemerella
Hydropsilus
Thraulus
L7
Baetis
Oecetis
Mystacides
Lestes
Caenis
Orectochilus
Oulimnius
Onychogomphus
Tabanidae
Sericostoma
Corynoneurinae
Calopteryx
Tipulidae
Simulidae
Ecdyonurus
1
Chironomini
Tanytarsini
Azabus
Elmis
Tanypodinae
Naididae
Calliarcys
Scyomyzidae
Ceratopogoninae
Lumbriculidae
Chaetopteryx
Sialis
L1
A
Nemoura
Leuctra
Pisidium
L2
Protonemura
0.7
-1.0
2.0
-1.5
B
L4
L3
L7
L2
L1
1.20
-0.03
0.25
0.00

Figure 5

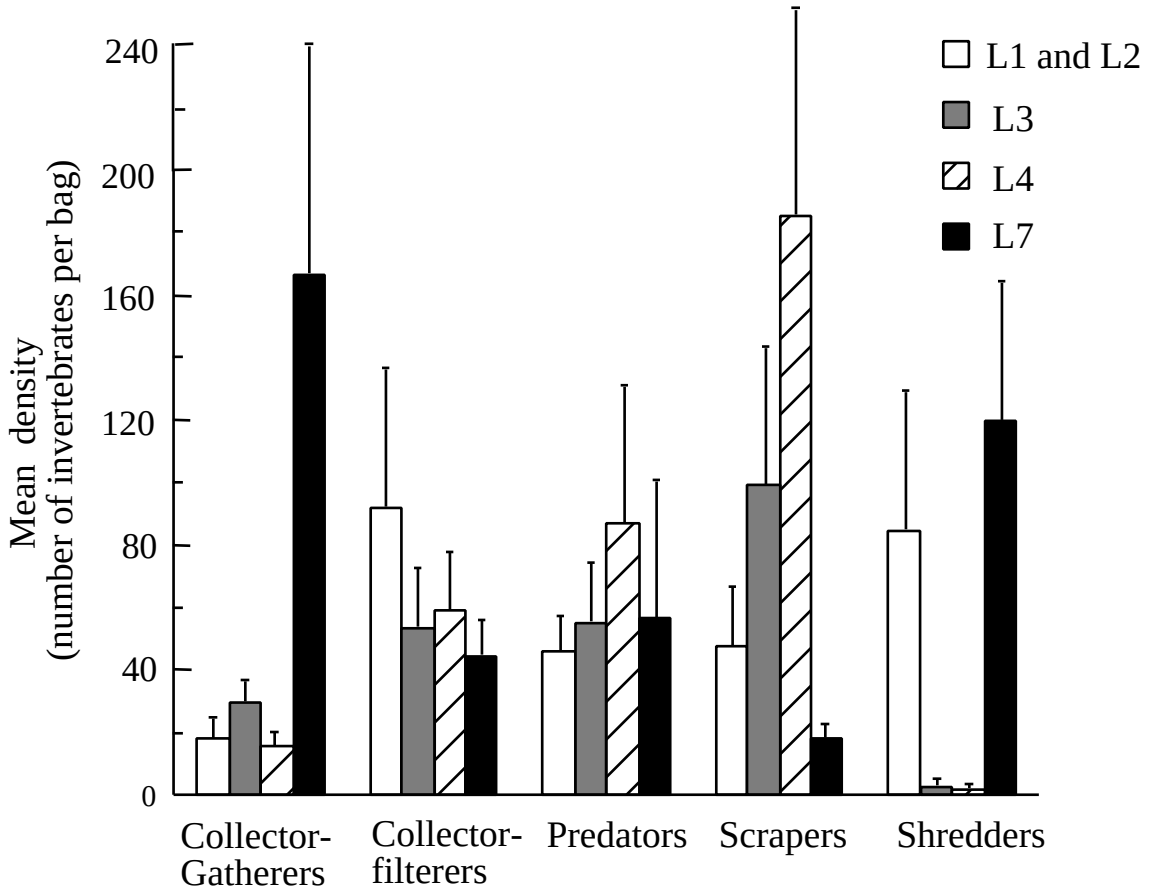
240
200
160
120
80
40
0
Mean density
(number of invertebrates per bag)
L1 and L2
L3
L4
L7
Collector-
Gatherers
Collector-
filterers
Predators
Scrapers
Shredders

Fig.6