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Comparison of two methods for estimating the abundance, diversity and habitat preference of fluvial macroinvertebrates in contrasting habitats

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ABSTRACT

In this research we evaluate the effects of the method used for estimating the potential surface available for benthic macroinvertebrates in macrophyte and unvegetated habitats on several metrics and habitat preference of aquatic macroinvertebrates in the upper catchment of the Henares River (Guadalajara, Central Spain). Three sampling sites were selected: a well-preserved stream (site A), a stream with no wood riparian vegetation (site B), and a straightened and deforested reach (site C). Two habitats were selected in each site: unvegetated habitat (i.e., substrata without macrophytes) and macrophyte habitat (i.e., substrata covered by macrophytes). In each habitat, six macroinvertebrate samples (including all macrophytes or mineral particles) were collected using a Hess sampler. Diversity and density of major families were referred to the surface of the Hess sampler (= Hess surface method) and to the actual surface of either mineral particles or macrophytes (= actual surface method). In general, for the actual surface method, biomass, richness, dominance, and diversity metrics were higher in the mineral habitat than in the macrophyte habitat. This trend was different for the Hess surface method. In general, densities turned out to be higher in the unvegetated habitat than in the macrophyte habitat when using the actual surface method, but the reverse occurred when using the Hess surface method. This fact is relevant for river biomonitoring, especially when reaches with different dominant substrates (macrophytes vs mineral) are compared using just one of the methods. It is concluded that the macrobenthic metrics and density values are influenced by the method used to estimate the potential available surface for aquatic macroinvertebrates.

Introduction

Macrophytes are an important component of fluvial ecosystems. They can modify the physical conditions of rivers and streams, such as current velocity, substrate and detritus type, increasing the heterogeneity of the habitat for aquatic macroinvertebrates (Hynes 1970; Gregg and Rose 1982, 1985; Ward 1992; Kaenel et al. 1998; Sand-Jensen 1998; Collier et al. 1999; Wetzel 2001; Allan and Castillo 2007). Therefore, the structure of the macroinvertebrate community and the habitat preference of species can be affected by the presence of macrophytes (Jenkins et al. 1984; Ormerod 1988; Ward 1992; Ó Hare and Murphy 1999; Wetzel 2001; Allan and Castillo 2007).

Gregg and Rose (1985) and Wright (1992) found that fluvial macroinvertebrate communities on macrophyte habitats had higher taxa richness than those on unvegetated areas. Several studies have showed that Chironomidae and Simuliidae are associated with macrophyte habitats (Percival and Whitehead 1929; Wright 1992; Kaenel et al. 1998; Kaenel and Uehlinger 1999). In contrast, Gregg and Rose (1985) found that the abundances of Simuliidae (*Simulium* sp.) and Chironomidae were higher in mineral areas than in vegetated areas. Rooke (1984) showed a higher abundance of macroinvertebrates on stones than on certain aquatic plants. These apparently contradictory findings may be attributed, among others, to the following two factors: (1) different methods used to estimate the available surface for macroinvertebrates may affect the final results and (2) different taxonomic groups may differ in their habitat preferences.

Regarding the method, the way of estimating the available surface for macroinvertebrates in different habitats, either in disturbed or in undisturbed reaches, may greatly affect the final taxa density assessment, but such effect has been ignored so far. In the literature there are two main groups of methods for estimating available surface for macroinvertebrates: those relating the abundance of macroinvertebrates to the sampling area of the device (such as Hess or Surber samplers) used to collect organisms from benthos (sampler surface methods), and those correcting the sampling area to account for stone surface and/or presence of macrophytes within the sampler (actual surface methods). The latter method normally uses the surface area/biomass relationships for macrophytes (Gregg and Rose 1982,

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1985; Armstrong et al. 2003), and sphere or ellipse formulas, or regressions of stone surface through the product of the greatest length by the greatest perimeter to estimate the stone surface (Calow 1972; Graham et al. 1988).

The aim of this research was to compare the influence of the method used for estimating the densities of benthic macroinvertebrates on the comparison of macroinvertebrate communities (i.e. several benthic metrics) occupying macrophyte and unvegetated habitats. Additionally, we assess the influence of method on habitat preferences of macroinvertebrates. We test the following two hypotheses: (1) the actual surface method would result in lower macroinvertebrate density in the macrophyte habitat than in the unvegetated habitat, as this method corrects for the larger available surface in the macrophyte habitat, (2) the density of particular macroinvertebrate groups (e.g. Elmidae, Ancylidae, Glossosomatidae, Limoniidae) would be higher in the unvegetated habitat than in the macrophyte habitat, while the reverse trend is expected for the family Hydroptilidae, which includes phytophagus species.

Materials and methods

The study area, sampling sites and selected habitats

The field study was carried out in the upper catchment of the Henares River (Guadalajara, Central Spain) (Fig. 1). The study section runs through limestone deposits at approximately 1000 m above the sea level. Three sampling sites were established along the study area. Site A was located in a reach of the Alboreca River, where the riparian vegetation was relatively well preserved (around 4 m in width) and dominated by poplar trees (*Populus* sp.). The streambed was dominated by gravel (44%) and boulders (25%). Site B was also located in the Alboreca River, about 1.8 km downstream from site A. The riparian vegetation was scarce, mainly composed by shrubs and grasses. The streambed was dominated by gravel (37%), boulders (31%) and sand-silt-fine sediment (31%). Finally, site C was located in the Henares River, about 4.9 km downstream from site A. This reach was straight as a consequence of anthropogenic activities (i.e., agricultural management), with the streambank vegetation being composed by grasses and emergent plants, and the streambed being dominated by sand, silt and fine sediment (98%).

Two habitats were selected in each sampling site: unvegetated habitat (i.e., substrata without macrophytes) and macrophyte habitat (i.e., substrata covered by submerged macrophytes). The

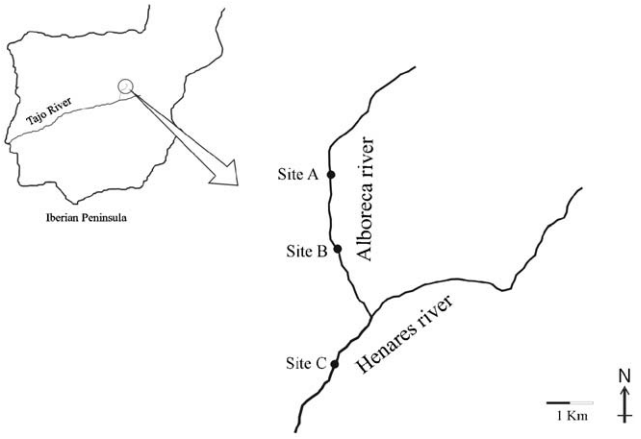

Fig. 1. Location of the upper catchment of Henares River (Guadalajara, Central Spain) showing sampling sites A, B, and C.

latter was dominated by the liverwort *Pellia endiviifolia* (Dicks.) Dum. in Site A, the moss *Drepanocladus aduncus* (Hedw) Warnst. in Site B, and the common stonewort *Chara vulgaris* L. in Site C. All these macrophytes grow close to the hard benthic substrate, forming relatively compact masses.

Sampling and laboratory procedures

Biological sampling was performed at the end of May 2002. In each sampling site (A, B, and C) and for each habitat (unvegetated habitat and macrophyte habitat), six benthic samples were taken using a modified Hess sampler (with a mesh size of 250 μm and enclosing a sampling area of 181.5 cm^2). In each sampling site a total of 12 benthic samples were taken (6 for macrophyte habitat and 6 for unvegetated habitat). In each sample, all mineral particles or macrophytes within the Hess sampler were collected to assess the actual surface available for macroinvertebrates. All mineral particles and macrophytes were collected using the net of the Hess sampler in order to avoid the loss of macroinvertebrates or habitat particles. In the case of macrophyte habitat, the Hess sampler was placed over the macrophyte mass until the sampler touched the hard bottom, then the whole mass of macrophytes (from the top of the hard benthic area to the top of the macrophyte mass) was removed. Each sample (macroinvertebrates plus mineral substrate or macrophytes) was placed in a plastic bottle and preserved with 4% formaldehyde solution. All macrobenthic metrics, main taxonomic groups, and families of macroinvertebrates (*see below*) were calculated on both bases (actual surface and Hess surface).

Water velocity was measured at 5–15 cm above the macrophyte and mineral substrate with a current meter (MiniAir 2 Schiltknecht) before to take each Hess sample. After collection in the field, samples were preserved in 4% formalin and packed for examination in the laboratory. Additionally, ten transects across each reach were also performed to identify the dominant submersed macrophyte, to assess the frequency of different types of macrophytes (submerged, emergent, and floating), and of different kinds of unvegetated substrata (sand, silt and fine sediment, gravel, coarse gravel, very coarse gravel, small cobbles, large cobbles, and boulders), and to characterize the physical properties of the reach (water velocity, water column depth, and wetted width). The methodology was based on Alonso (2005), and the procedure was as follows: in each transect ten specific measurements of the presence of macrophytes or mineral substrate, water velocity (MiniAir 2 Schilknecht), and water column depth were monitored. The total number of measurements per sampling site was 100, which allow calculating the frequency of macrophytes and unvegetated substrate, and the mean ($n = 100$) water velocity and water column depth. The wetted width of each transect was measured to calculate the mean ($n = 10$) wetted width of each sampling site. Finally, dominant submersed macrophyte samples were taken to the laboratory for accurate taxonomic determination.

During April, May and June of 2002, maximum and minimum water temperature (max/min thermometer), dissolved oxygen concentration (Oxymeter Crison Oxi-320), and water conductivity (Conductimeter Crison 524) were also measured in each sampling site. In addition, water samples were collected to analyse total alkalinity, pH, nutrient (NH_4-N, NO_2-N, NO_3-N and PO_4-P) concentrations, and chloride (Cl^-) and calcium (Ca^{2+}) content, following the standardized methods described by American Public Health Association (1995).

Once in the laboratory, macroinvertebrates were separated from macrophytes or from mineral substrate: macroinvertebrates were sieved using 4 stainless steel sieves (minimum mesh size of

250 μm), and habitat particles were washed on the top of the sieve column using tap water in order to remove all macroinvertebrates. All removed macroinvertebrates were identified mostly at genus-species level; family level was used only for Diptera (except for Simuliidae) and Oligochaeta. Then, individuals of each taxon were sorted together, counted and then dried for 72 h at 60 °C to determine their dry biomass. Macrophyte area was calculated from surface area/biomass relationship (Gregg and Rose 1982, 1985; Armstrong et al. 2003) for each species and sample. The linear regression equations for the ash-free dry mass (g) ($= X$) versus surface (cm^2) ($= Y$) were $Y = 644.2X$ for *P. endiviifolia*, $Y = 551.3X$ for *D. aduncus* and $Y = 219.4X$ for *C. vulgaris*. Total mineral area (mineral particles higher than 1 cm) was determined approaching the stones to sphere (Graham et al. 1988). The formula of area $= 4\pi r^2$ was used substituting r^2 by $(LW+LH+WH)/12$, where LW = length × width, LH = length × height and WH = width × height of stones. The estimated error of this method is ±9.5% (Dall 1979). The half of total stone surface was considered as the exposed stony area available for macroinvertebrate's colonization (Gregg and Rose 1985). When the calculated mineral area had lower than sampler surface, Hess area (181.5 cm^2) was used. It was frequent at site C, where substrate was dominated by fine particles (sand, silt, and fine sediment).

Macrobenthic metrics

Different macrobenthic metrics were used to assess the structure of the macroinvertebrate community: total density (total individuals/m^2), total biomass (mg dry weight/m^2), taxa richness (total number of taxa/cm^2), EPT richness (number of Ephemeroptera, Plecoptera, and Trichoptera taxa/cm^2), EPTC richness (number of Ephemeroptera, Plecoptera, Trichoptera, and Coleoptera taxa/cm^2), and taxa dominance and diversity per cm^2. Dominance (d) and diversity (D) were calculated using the following Camargo (1992, 1995) indices:

$$\text{Dominance } (d) = \sum_{d=1}^{L} (p_d - 1/S)$$

and

$$\text{Diversity } (D) = S - (S \times d)$$

where S is the number of species or taxa in the community, L is the number of dominant species or taxa, and p_d is the relative abundance of each dominant species or taxon (a species or taxon is dominant if its relative abundance $> 1/S$, and subordinate if its relative abundance $< 1/S$). The product $(S \times d)$ represents the impact that dominance causes on the maximum possible value of diversity (i.e., species richness).

We calculated the density of each major taxonomic group (Amphipoda, Coleoptera, Diptera, Ephemeroptera, Mollusca, Oligochaeta, Plecoptera, Trichoptera, Tricladida) and each taxonomic family (number of individuals per m^2) in each habitat and sampling site.

Statistical analyses

The effect of site on the physicochemical parameters was assessed through a one-way ANOVA followed by a Tukey test (Zar 1984). Data were tested for heterogeneity of variance using Levene's test (Levene 1960), and when necessary, data were log-transformed to achieve homoscedasticity. A level of $P < 0.05$ was chosen for ANOVA and Tukey tests. Mean values of each macrobenthic metric, principal taxonomic group, and family were compared between habitats (macrophyte versus unvegetated) through a Mann–Whitney U-test (Zar 1984) for each sampling site and method (Hess surface or actual surface). A significance level of $P < 0.01$ was selected to reduce the probability of committing type I error (Toft and Shea 1983; Rotenberry and Wiens 1985). To compare the whole community composition (at family level) between habitats for each method and site, a multi-response permutation procedure (MRPP) was conducted (Zimmerman et al. 1985; McCune et al. 2002). This method is a nonparametric multivariate analysis that permits testing the null hypothesis of no treatment effect on the taxonomic composition of the samples. MRPP is based on the within-group average of pairwise distance measures between object response values in a Euclidian data space (Zimmerman et al. 1985). For this test a significant P-value < 0.01 was chosen. All statistical univariate analyses were performed using SPSS 11.5, and the multivariate analyses using PC-ORD 4.0 software.

Results

Environmental and physicochemical variables

The lowest velocity, higher depth, and wetted width were found in site C (Table 1). Mineral substrate in site C was dominated by sand, silt, and fine sediment, in site B by boulders and sand, silt, and fine sediment, and in site A by boulders. The highest coverage of macrophytes was found in sites B and C.

Mean physicochemical parameters for each sampling site are shown in Table 2. No significant differences between sites were found for nitrate, nitrite, ammonia, and calcium concentrations ($P > 0.05$; Tukey test). For the rest of the physicochemical parameters shown in Table 2, significant differences were found between sampling sites (ANOVA; Tukey test; $P < 0.05$). Water velocity did not significantly differ between the two habitats in each sampling site (data not shown).

Macrobenthic metrics

For the actual surface method, density, biomass, richness, dominance, and diversity metrics were significantly higher in the mineral habitat than in the macrophyte habitat for all sampling

Table 1
Mean values (±SD) of water velocity ($n = 100$), depth ($n = 100$), and wetted width ($n = 10$) for each sampling site.

	Site A	Site B	Site C
Mean physical parameters			
Water velocity (cm/s) ($n = 100$)	14.9±13.5	23.1±25.9	9.5±9.3
Depth (cm) ($n = 100$)	8.8±6.0	10.9±6.6	13.8±8.9
Wetted width (m) ($n = 10$)	1.8±0.3	1.2±0.5	2.8±0.3
Mineral substrate cover (%)			
Sand, silt, and fine sediment (<2 mm)	16	31	98
Gravel (2–16 mm)	18	24	0
Course gravel (16–32 mm)	11	6	1
Very course gravel (32–64 mm)	15	7	0
Small cobbles (64–128 mm)	14	1	1
Large cobbles (128–256 mm)	1	0	0
Boulders (>256 mm)	25	31	0
Macrophyte cover (%)			
Without macrophytes	76	34.6	39.7
Submersed	24	34.6	14.4
Emergent	0	30.8	35.1
Floating	0	0	10.8

The relative frequencies of stony substrate and macrophyte type at each sampling site are shown (sampling sites see Fig. 1).

Table 2
Mean ($n = 3$) values ± standard deviations of physicochemical parameters in each sampling site (sampling sites see Fig. 1).

	Site A	Site B	Site C
Total alkalinity (mg $CaCO_3$/L)	274.6 ± 7.5 [b]	268.3 ± 5.6 [b]	236.3 ± 13.2 [a]
Conductivity (μS/cm)	533 ± 2.2 [a]	564 ± 18.1[a]	955 ± 100.4 [b]
Max water T[a]–Min water T[a] (°C)	5.3 ± 0.6 [a]	10.3 ± 1.2 [b]	12.7 ± 2.1 [b]
pH	7.6 ± 0.0 [a]	8.1 ± 0.1 [b]	8.1 ± 0.0 [b]
Dissolved oxygen (mg O_2/L)	9.2 ± 0.3 [a]	10.5 ± 0.4 [b]	13.8 ± 0.6 [c]
Nitrate (mg NO_3-N/L)	1.8 ± 0.7	2.0 ± 0.8	1.7 ± 0.4
Nitrite (mg NO_2-N/L)	0.008 ± 0.008	0.011 ± 0.008	0.009 ± 0.012
Total ammonia (mg NH_4-N/L)	0.032 ± 0.010	0.056 ± 0.011	0.122 ± 0.163
Phosphate (mg PO_4-P/L)	<0.010	<0.010	<0.010
Calcium (mg Ca^{2+}/L)	73.5 ± 16.6	102.1 ± 16.1	108.5 ± 16.1
Chloride (mg Cl^-/L)	6.3 ± 0.6 [a]	8.2 ± 0.5 [a]	121.8 ± 16.9 [b]

Concentrations of phosphate were below detection limits (<0.010 mg/L PO_4-P). Different letter means significant differences between sampling sites for each parameter (ANOVA; Tukey test; $P < 0.05$). Sampling sites with the same letter did not differ significantly in the physicochemical parameter (ANOVA; Tukey test; $P > 0.05$).

sites (Mann–Whitney *U*-test; $P < 0.01$), except for total density (site B) and total density and biomass (site C) (Table 3). This trend was different for the Hess surface method: in site A Camargo's diversity was higher in the mineral habitat, whereas Camargo's dominance was lower in the mineral habitat; in site B, total density showed a higher value in the macrophyte habitat; and in site C, total density and biomass were higher in the macrophyte habitat.

Habitat preference

In site A, with both methods, Oligochaeta and Trichoptera groups and the families Elmidae (*Elmis* sp., *Riolus* sp., *Limnius volckmari*, *Esolus* sp. larvae and adults) Ancylidae (*Ancylus fluviatilis*), Naedidae-Tubificidae, Glossosomatidae (*Synagapetus* sp.), and Psychomyiidae (*Tinodes* sp.) showed preference for the unvegetated habitat (Table 4). Coleoptera, Diptera, Mollusca, and Plecoptera groups and the families Chironomidae, Bythinellidae (*Bythinella* sp.), and Nemouridae (*Protonemura* sp.) showed different results according to the method used (Table 4).

In site B the Diptera group and the families Hydraeniidae (*Hydraena* sp.), Chironomidae and Hydroptilidae (*Ithytrichia* sp. and *Hydroptila* sp.) showed preference for the macrophyte habitat irrespective of the sampling method (Table 4). Simuliidae (*Simulium* sp.), Stratyomidae and Lumbriculidae-Enchytraeidae showed a similar preference with both methods. According to the method used, preference or no preference for a specific habitat was different for Amphipoda, Coleoptera, and Ephemeroptera groups, and for families Elmidae (*Elmis* sp., *Riolus* sp., *Limnius volckmari*, *Esolus* sp. larvae and adults), Empididae and Baetidae (*Baetis rhodani*).

In site C, the same preference for the macrophyte habitat, independent of the method used, was shown by the Trichoptera group and the families Baetidae (*B. rhodani*) and Hydroptilidae (*Ithytrichia* sp.) (Table 4). The dipteran Limoniidae showed preference for the unvegetated habitat with both methods.

The multivariate analysis (MRPP) showed significant differences in the community composition between habitats in sites A and B for both methods ($P < 0.01$; MRPP test) (Table 5). In site C, the same was true for the Hess method, while the actual surface method showed no significant habitat effect.

In summary, seven, six, and three taxa showed significant differences between methods for sites A, B, and C, respectively.

Table 3
Mean values (±SD) for macrobenthic metrics in each site, calculated with different methods (Hess and Actual).

Metrics	Site A				Site B				Site C			
	Hess		Actual		Hess		Actual		Hess		Actual	
	MA	UN	MA	UN	MA	UN	MA	UN	MA	UN	MA	UN
Total density (individuals/m^2)	47530 ± 11950	41625 ± 16291	8228 ± 1536	**32029*** ± 15005	**172424*** ± 63506	57080 ± 17118	31117 ± 16087	50389 ± 15730	**121506*** ± 18581	43728 ± 30846	38740 ± 5741	43738 ± 30853
Total biomass(mg dw/m^2)	8880 ± 2591	6929 ± 3089	1524 ± 294	**5358*** ± 2719	21871 ± 11725	18133 ± 4738	4019 ± 2755	**16117*** ± 4813	**10447*** ± 2403	3054 ± 1928	3363 ± 983	3055 ± 1929
Richness(notaxa/cm^2)	0.109 ± 0.016	0.113 ± 0.009	0.019 ± 0.004	**0.085*** ± 0.012	0.129 ± 0.014	0.118 ± 0.020	0.023 ± 0.007	**0.103*** ± 0.013	0.082 ± 0.006	0.082 ± 0.011	0.026 ± 0.005	**0.082*** ± 0.011
EPT richness(notaxa EPT/cm^2)	0.032 ± 0.006	0.032 ± 0.005	0.006 ± 0.001	**0.024*** ± 0.003	0.046 ± 0.003	0.038 ± 0.013	0.008 ± 0.002	**0.033*** ± 0.011	0.030 ± 0.004	0.023 ± 0.005	0.010 ± 0.003	**0.023*** ± 0.005
EPTC richness (notaxa EPTC/cm^2)	0.053 ± 0.006	0.062 ± 0.007	0.010 ± 0.002	**0.047*** ± 0.007	0.074 ± 0.007	0.062 ± 0.012	0.013 ± 0.004	**0.055*** ± 0.009	0.045 ± 0.005	0.043 ± 0.006	0.015 ± 0.004	**0.043*** ± 0.006
Camargo's dominante (d/cm^2)	**0.004*** ± 0.0002	0.003 ± 0.0003	0.001 ± 0.0002	**0.002*** ± 0.0003	0.004 ± 0.0001	0.004 ± 0.0003	0.001 ± 0.0002	**0.003*** ± 0.0007	0.003 ± 0.0002	0.003 ± 0.0006	0.001 ± 0.0002	**0.003*** ± 0.0005
Camargo's diversity(D/cm^2)	0.031 ± 0.004	**0.046*** ± 0.004	0.006 ± 0.002	**0.035*** ± 0.008	0.034 ± 0.004	0.038 ± 0.010	0.006 ± 0.002	**0.033*** ± 0.007	0.037 ± 0.003	0.034 ± 0.007	0.012 ± 0.002	**0.034*** ± 0.007

Asterisk shows significant differences between habitats (MA = macrophyte and UN = unvegetated) for each method and site (Mann–Whitney *U*-test; $P < 0.01$). The highest significant value in each pair is shown in boldface (sampling sites see Fig. 1)

Table 4
P values for the comparison of the main groups and families between habitats (MA = macrophyte and UN = unvegetated) in each site and with each method (Hess and Actual).

	Site A		Site B		Site C	
	Hess	Actual	Hess	Actual	Hess	Actual
	MA UN	MA UN	MA UN	MA UN	MA UN	MA UN
Amphipoda-Gammaridae	0.015 (>)	n.s	n.s	**0.006 (<)**	n.s	n.s
Coleoptera	n.s	**0.004 (<)**	n.s	**0.004 (<)**	n.s	n.s
Elmidae	**0.004 (<)**	**0.004 (<)**	n.s	**0.004 (<)**	n.s	n.s
Gyrinidae	0.021 (<)[a]	0.022 (<)[a]	n.s	n.s	n.s	n.s
Hydraeniidae	n.s[b]	n.s[b]	**0.006 (>)**[b]	0.007 (>)[b]	n.s[a,b]	n.s[a,b]
Scirtidae	n.s	0.025 (<)	n.s	n.s	n.s[a,b]	n.s[a,b]
Diptera	n.s	**0.004 (<)**	**0.004 (>)**	0.004 (>)	**0.004 (>)**	n.s
Chironomidae	n.s	**0.004 (<)**	**0.004 (>)**	**0.004 (>)**	**0.004 (>)**	n.s
Simuliidae	n.s[b]	n.s[b]	**0.003 (>)**	0.010 (>)	n.s[b]	n.s[b]
Stratyomyidae	n.s	n.s	**0.004 (>)**	0.010 (>)	n.s[a,b]	n.s[a,b]
Limoniidae	n.s	n.s	n.s	n.s	**0.002 (<)**[a]	0.002 (<)[a]
Empididae	n.s	n.s	**0.006 (>)**	n.s	n.s[a]	n.s[a]
Ephemeroptera	0.046 (>)	n.s	n.s	**0.004 (<)**	0.004 (>)	0.010 (>)
Baetidae	n.s	n.s	n.s	**0.004 (<)**	**0.004 (>)**	**0.004 (>)**
Caenidae	n.s	n.s	n.s	n.s	**0.004 (>)**	n.s
Ephemerellidae	n.s[a]	n.s[a]	0.018 (>)	n.s	n.s	n.s
Mollusca	n.s	**0.004 (<)**	n.s	0.010 (<)	0.025 (>)	n.s
Ancylidae	**0.004(<)**	0.003 (<)	n.s[a]	n.s[a]	n.s[a,b]	n.s[a,b]
Bythinellidae	n.s	**0.004 (<)**	n.s	0.010 (<)	n.s[a,b]	n.s[a,b]
Hydrobiidae	n.s[a,b]	n.s[a,b]	n.s[a,b]	n.s[a,b]	0.030 (>)	n.s
Sphaeridae	n.s	n.s	n.s	n.s	0.022 (>)[b]	0.022 (>)[b]
Oligochaeta	**0.004(<)**	**0.004(<)**	n.s	n.s	0.010 (>)	n.s
Lumbriculidae-Enchytraeidae	n.s	n.s	0.016 (<)	0.013 (<)	0.010 (>)	n.s
Naedidae-Tubificidae	**0.004(<)**	0.004(<)	0.045 (>)	n.s	0.037 (>)	n.s
Plecoptera	n.s	**0.004(<)**	n.s	n.s	n.s[a,b]	n.s[a,b]
Nemouridae	n.s	**0.004(<)**	n.s[b]	n.s[b]	n.s[a,b]	n.s[a,b]
Perlodidae	n.s	n.s	0.049 (>)	n.s	n.s	n.s
Trichoptera	**0.006(<)**	**0.004(<)**	0.010 (>)	n.s	**0.004 (>)**	0.004 (>)
Glossosomatidae	**0.006 (<)**	0.004(<)	n.s[a]	n.s[a]	n.s[a,b]	n.s[a,b]
Hydroptilidae	n.s	n.s	**0.003 (>)**	**0.005 (>)**	**0.004 (>)**	0.004 (>)
Psychomyiidae	**0.005 (<)**	**0.003 (<)**	n.s	0.036 (<)	n.s[a,b]	n.s[a,b]
Rhyacophiloidae	n.s	n.s	0.024 (>)	n.s	n.s[a,b]	n.s[a,b]
Triclades-Planariidae	n.s	0.013 (<)	0.025 (>)	n.s	n.s[a]	n.s[a]

P values in boldface are considered to be significant (Mann–Whitney U-test; $P<0.01$). Signs '<' and '>' show the habitat with the higher value for each parameter (sampling sites see Fig. 1).

[a] Absent in macrophyte.
[b] Absent in unvegetated habitat.

Table 5
Summary statistics for MRPP between habitats (macrophyte and unvegetated habitats) for each site and method (sampling sites see Fig. 1).

Site	Method	*P*	*A*
A	Hess	**0.0056**	0.2017
A	Actual	**0.0018**	0.2538
B	Hess	**0.0005**	0.3786
B	Actual	**0.0021**	0.3398
C	Hess	**0.0061**	0.1878
C	Actual	0.1135	0.0567

P values in boldface are considered to be significant. The *A* value is the chance-corrected within-group agreement, and it describes the within-group homogeneity, compared to the random expectation (McCune et al., 2002).

The whole community did not differ between habitats in site C on the basis of the actual method, while in the rest of the sampling sites there was a significant effect of habitat on community with both methods.

Discussion

Environmental and physicochemical variables

The environmental properties of sampling sites showed that the reach with the least mineral substrate complexity was found at site C, as it was dominated by fine particles (<2 mm of diameter). That is a characteristic of straight or canalized streams, where the absence of riparian vegetation increases the fine sediment stream-input from riparian lands (Wood and Armitage 1997; Henley et al. 2000; Landwehr and Rhoads 2003). This process, together with the higher light radiation – as a consequence of the lack of riparian vegetation – can produce an increase of submersed macrophytes, modifying several physico-chemical properties of the stream (Hynes 1970; Gregg and Rose 1982, 1985; Sand-Jensen 1998; Collier et al. 1999). Higher densities of aquatic macrophytes can also reduce the flow velocity, increasing the accumulation of fine particulates in the benthic zone. The higher concentrations of dissolved oxygen in sites B and C as compared to site A may be explained by the higher frequency

of macrophytes in both sites, which can modify the dissolved oxygen regimens as a consequence of photosynthetic activity (Carpenter and Lodge 1986; Dudley et al. 1986).

Macrobenthic metrics

The present study shows that the comparison of macroinvertebrate community structure between macrophyte and mineral habitat is affected by the method used to estimate the potential available area for invertebrates (i.e. total surface that has been measured in each kind of habitat with each method). For the actual surface method, macrophytes provide a higher surface than the unvegetated habitat (Gregg and Rose 1985). This fact could explain the higher values of density, biomass, richness, and diversity metrics found in the unvegetated habitat than in the macrophyte habitat in all sampling sites. By contrast, the sampler surface method gives the same surface to both habitats, reducing differences between them. Gregg and Rose (1985) compared the macroinvertebrate community between experimental trays differing in substrate using the actual surface, finding higher invertebrate density and diversity in unvegetated habitats. On the contrary, other studies based on the sampler surface generally found higher taxa richness and densities on the macrophyte habitat (Wright 1992; Ó Hare and Murphy 1999). In our study, the sampler surface method showed a higher invertebrate density in the macrophyte habitat than in the unvegetated habitat for sites B and C but little differences regarding diversity metrics.

Habitat preference

This study has shown that some taxa were strongly associated with either unvegetated habitat or macrophyte habitat, though such associations usually appeared in just one of the sampling sites and depended on the method used. Other studies have shown preference to the same families of site A for the unvegetated habitat (Elmidae: Armitage and Cannan 2000, Ancylidae: Percival and Whitehead 1929, Glossosomatidae: Percival and Whitehead 1929; Minshall and Minshall 1977; Gregg and Rose 1985; Wright 1992, and Psychomyiidae: Percival and Whitehead 1929). A possible cause of the Elmidae preference for the unvegetated habitat in site A may be the higher roughness of this habitat, which allows both larvae and adults to cling more easily than in the flat and smooth leaves of *P. endiviifolia*. In the case of the stream limpet *A. fluviatilis*, which is a scraper mollusc that feeds on periphyton, the mineral substrate should provide more food than the macrophyte substrate (Hynes 1970; Tachet et al. 2000). Larvae of Glossosomatidae and Psychomyiidae need coarse sand and fine gravel-sand grains to make their cases, and they are easier to find in the unvegetated substrate (Percival and Whitehead 1929). Moreover, larvae of Glossosomatidae need a hard surface in order to graze on epilithic algae (Wright 1992). In the present study, *P. endiviifolia* grows close to the substrate forming a rosette, with smooth, flat, and lobulated leaves. These traits have been shown to influence the habitat preference of aquatic invertebrates (Rooke 1984, 1986; Cheruvelil et al. 2000; Bolam and Fernandes 2002). The lack of dissected and rough leaves of *P. endiviifolia*, and the higher heterogeneity of the unvegetated substrate in site A, could explain the lack of preference for the macrophyte habitat.

In the case of site B, Hydraenidae, Chironomidae, Simuliidae, Stratyomyidae, and Hydroptilidae families preferred the macrophyte habitat independent of the method. Hydroptilidae, Chironomidae, and Simuliidae have been found to associate with the macrophyte habitat by many authors (Percival and Whitehead 1929; Rooke 1986; Wright 1992; Kaenel et al. 1998; Kaenel and Uehlinger 1999; Armitage and Cannan 2000). However, the reverse trend has been reported by Gregg and Rose (1985) for Simuliidae and Chironomidae. Percival and Whitehead (1929) found high densities of Hydroptilidae (*Hydroptila* sp. and *Ithytrichia* sp.) in the macrophyte habitat. Among these authors, only Gregg and Rose (1985) used the actual surface method. The dipterans of the family Chironomidae may benefit from macrophytes for feeding, refuge, or to avoid predation (Percival and Whitehead 1929; Rooke 1986; Newman 1991). Larvae of Simuliidae may benefit from macrophytes because the pattern of rapid flow over their surfaces could be a favourable microenvironment for the larvae of Simuliidae (Kaenel et al. 1998; Armitage and Cannan 2000). Larvae of family Stratyomidae are a collector–gatherer that feeds on fine particulate organic matter (FPOM) (Davis et al. 2001; Allan and Castillo 2007). *D. aduncus* is a macrophyte with finely dissected leaves that could accumulate more FPOM than other broad-leaf plants (as *P. endiviifolia*) or unvegetated habitat, which could explain the high density of Stratyomidae on this macrophyte. The piercing caddis larvae *Hydroptila* sp. (family Hydroptilidae) use macrophytes as a direct source of food and for this reason it has been found in vegetated areas (Dudley et al. 1986).

In the case of site C (dominated by sand, silt, and fine sediment), the density and biomass of macroinvertebrates tended to be higher in the macrophyte habitat. It is known that small- to medium-sized stones harbour more invertebrates than softer substrates (Hynes 1970; Cummins 1973; Rooke 1984). In reaches where macrophytes grow in soft substrate, they represent the most stable habitat and they may increase densities of macroinvertebrates as compared with immediate substrate (Rooke 1984). The mayfly *B. rhodani* can have a preference for macrophytes, due to its higher stability, the provision of refuge, and as an indirect source of food. Limoniidae showed preference for unvegetated habitat, this diptera being a burrower that dwells in fine sediment (Tachet et al. 2000).

In general, the major cause of differences between methods may be that potential available area (i.e. total measured surface) in macrophytes does not necessarily coincide with the colonized area (i.e. total surface taken up by invertebrates), the latter being lower than the surface measured by regression (Gregg and Rose 1982, 1985; Armstrong et al. 2003). A clear example of that is Simuliidae family, whose larvae usually colonize the upper part of macrophytes, which are exposed to higher current velocities. In this case, the actual surface measured by regression can overestimate the actual colonized surface. By contrast, the sampler surface method does not consider the higher surface available for other generalist invertebrates (i.e. Chironomidae) in the macrophyte habitat. Therefore it could overestimate the density, biomass, richness, and diversity of macroinvertebrates on that habitat (Percival and Whitehead 1929; Whitehead 1935; Barber and Kevern 1973; Wright 1992; Ó Hare and Murphy 1999).

In general, the whole community responded in a similar way than the individual taxa, as the communities were different between most of the sites and habitats. This shows that the community structure is highly dependent on the habitat, and in sites with a poor complex benthic zone it is also dependent on the method used to estimate the density of the macroinvertebrates.

Conclusions

The present study has shown that the comparison of macrobenthic metrics between the macrophyte habitat and the unvegetated habitat can be influenced by the method used to estimate the potential available surface for macroinvertebrates. In general, the actual surface method tends to overestimate the

surface for macroinvertebrates living in the macrophyte habitat, especially those groups that cannot colonize all potential available surfaces (e.g. simulids). The sampler surface method tends to overestimate the density, biomass, richness, and diversity of macroinvertebrates in the macrophyte habitat, especially for invertebrates that can colonize all macrophyte surfaces. Further knowledge is necessary on the colonization potential of different habitats by different macroinvertebrate to get an accurate estimation of the actual available surface for macroinvertebrates. Additionally, we recommend a careful selection of the method to estimate the potential available surface for macroinvertebrates during biomonitoring studies. This is especially relevant when reaches with strong differences in the benthic substrate are compared (e.g. macrophyte-dominated habitat vs mineral habitat). In the case of generalist groups (i.e. macroinvertebrates that are able to colonize all available surface), the actual method is recommended, while the sampler method may be suitable for non-generalist taxa (i.e. Simullidae), since the sampler surface can be more similar to the real colonized surface.

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