



ISSN: 0270-5060 (Print) 2156-6941 (Online) Journal homepage: www.tandfonline.com/journals/tjfe20

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To cite this article: Avaro Alonso & Julio A. Camargo (2005) Evaluating the Effectiveness of Five Mineral Artificial Substrates for the Sampling of Benthic Macroinvertebrates, Journal of Freshwater Ecology, 20:2, 311-320, DOI: 10.1080/02705060.2005.9664971

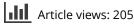
To link to this article: https://doi.org/10.1080/02705060.2005.9664971



Published online: 11 Jan 2011.



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Evaluating the Effectiveness of Five Mineral Artificial Substrates for the Sampling of Benthic Macroinvertebrates

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ABSTRACT

We evaluated the effectiveness of five artificial substrates to recreate the natural benthic macroinvertebrate community in an upper reach of the Henares river (Guadalajara, Spain). Five different substrates were built with natural mineral materials from the same reach. These included small, medium and large stones, with mean maximum diameters of 1.9, 4.9 and 9.0 cm, respectively, plus a mixture of three stone sizes and a treatment without stones (slab). Four replicates of each substrate were left in the stream for 30 days, and then they were removed for community analysis. The natural community was sampled with a Hess sampler near the experimental substrates. No significant differences were found between treatments for several community metrics (taxa richness, EPT richness, EPT density, diversity and dominance indices, and two biotic indices). However, densities of particular taxa were affected by treatments; Coleoptera and Diptera densities were the highest in the natural community and in the small stone treatment, respectively. Some families showed differences between treatments. The highest Elmidae and Scirtidae densities were found in the natural community. Medium stone treatment had the highest density of Simuliidae, while that of Chironomidae was the highest in the small stone treatment. The smallest density of Glossosomatidae was found in slab treatment. The macroinvertebrate communities colonizing the artificial substrates were similar to the natural one, although the densities of some groups varied.

INTRODUCTION

Artificial substrates have been widely used and recommended to study benthic macroinvertebrate communities in freshwater ecosystems (Rosenberg and Resh 1982; Platts et al. 1983, Casey and Kendall 1996 and 1997, Paller and Specht 1997, Humphries et al. 1998, Barbour et al. 1999, Grumiaux et al. 2000, Jesus et al. 2001). This methodology has several advantages over direct sampling of natural substrate, namely standardization of the sampling conditions, reduction of natural variability, and reduction of skill and training efforts (De Pauw et al. 1986, Hellawell 1986, Barbour et al. 1999). On the other hand, this sampling methodology may not represent the natural benthic assemblage properly, as artificial substrates may not offer the same microhabitats as natural substrates. Therefore, both community structure and density of certain taxa can be altered (Casey and Kendall 1996, Barbour et al. 1999).

Artificial substrates are usually built with different materials, such as PVC, bricks, concrete blocks, snags, turf, leaves or natural pebbles (Rutherford 1995, Casey and Kendall 1997, Humphries et al. 1998, Aikins and Kikuchi 2001, Carter and Resh 2001, Daugherty and Juliano 2001, Koperski 2003). When substrates recreate natural conditions (microhabitats, mineral composition), after an appropriate period for colonization, the macroinvertebrate community structure in artificial substrates can be very similar to that in the natural stream bottom (Rosser and Pearson 1995). However, the assemblages colonizing experimental substrates can be different from those of natural

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sampling when artificial materials are used to build them. Sometimes taxa density and richness are higher in natural substrates, as reported by Casey and Kendall (1996, 1997) and McCabe and Gotelli (2003), while Cascorbi (2002) reported a reverse trend. Shaw and Minshall (1980) found a similar number of taxa between Hess samples and trays filled with uniform-sized pebbles. These discrepant results evidence the necessity for evaluating the effect of the substrate nature on the macrobenthic sampling assessment (Casey and Kendall 1996 and 1997).

The objective of the present study was to evaluate the differences between benthic macroinvertebrate communities colonizing five artificial substrates, built with natural mineral rocks, and the natural assemblage in a reach of the Henares River, Spain. Additionally, we assessed the effectiveness of artificial substrates in the biomonitoring of the stream ecological quality. Our hypothesis was that artificial substrates built with natural rocks from the same river would be highly effective in recreating the structure and taxonomic composition of the natural community of benthic macroinvertebrates.

METHODS AND MATERIALS

The study was carried out in an upper reach of the Henares River (Guadalajara Province, Spain). The upper section of this stream runs through limestone deposits at approximately 1000 m above the sea level. The riparian vegetation is dominated by poplar (*Populus* × *euroamericano*). The stream bottom was dominated by gravel (44%) and boulders (25%). The average wetted width and depth of this reach were 2.1 and 0.12 m, respectively. Discharge at the time of sampling (June 2002) was 0.04 m³ s⁻¹. Mean (n=3) water physicochemical properties (±SD) at the sampling time were 8.3±0.8 mg O₂/L for dissolved oxygen, 14.7±0.6 °C for maximum temperature, 11.3±1.2 °C for minimum temperature, 529.7±6.5 μ S/cm for conductivity, 7.6±0.1 for pH, 6.5±0.6 mg Cl⁻/L for chloride and 280.1±12 mg CaCO₃/L for alkalinity.

Five different artificial substrates were built. Baked clay plates with a maximum diameter of 23 cm and an edge height of 2.2 cm were used as bases. Each plate, except those used in the slab treatment, was filled with 246 g of dry sand (diameter<1 mm). Different size stones were collected from the Henares River, cleaned of organic matter and invertebrates with a plastic brush, dried at 60°C for 48 hours, and subsequently placed in each plate. The five treatments were small, medium, large, mixture, and slab. Mean maximum diameters of stones for small, medium, and large treatments were 1.9, 4.9, and 9.0 cm, respectively. The mixture treatment was a combination of the three sizes. and the slab treatment was a plate without stones or sand. Each substrate was enclosed in a plastic net of 1 cm mesh. Four replicates were used for each treatment, although one of the slab replicates was lost during the colonization period. Artificial substrates were randomly placed on the streambed in riffle areas. After 30 days of colonization, substrates were retrieved with a hand-net (mesh size = 0.250 mm) moved against the stream. Invertebrates were removed from artificial substrates with a soft brush at the riverside and preserved in 4% formalin. Additionally, four riffle samples of benthic macroinvertebrates were collected on the natural mineral bed using a Hess sampler with a mesh of 0.250 mm (=Hess treatment). Hess and artificial substrate surfaces were 433.7 cm² and 433.4 cm², respectively. On three occasions during the colonization period, the water velocity over the substrates were measured using a flowmeter (MiniAir2 Schilknecht). This parameter was also measured in the Hess samples. Detritus (leaves, dead macrophytes, branches, and roots) accumulated in each artificial substrate and collected in the Hess samples were dried at 60°C for 72 hours. No significant differences were found between treatments for mean water velocity and for mean dry weight of detritus (Kruskal-Wallis or ANOVA test, p>0.05). In the laboratory, invertebrates were counted and identified mostly at genus-species level; family level was used only for Diptera (except for Simuliidae) and Oligochaeta.

Several macrobenthic metrics were calculated to assess the structure of the macroinvertebrate community: total density (total individuals/m²), richness (total number of taxa/m²), EPT richness (number of Ephemeroptera, Plecoptera and Trichoptera taxa/m²), EPT density (total number of individuals of these taxa/m²), Camargo's (1992) dominance index (d) and Camargo's (1992) diversity index (D). In addition, Camargo's biotic indices (t-BMWQ and a-BMWQ) were applied to examine the effect of artificial substrates on the biological monitoring of water quality based on benthic macroinvertebrates. The biological monitoring water quality (BMWQ) system is based on the British BMWP score system (Armitage et al. 1983) and was adapted for the biological monitoring in freshwater rivers of the Iberian Peninsula (Camargo 1993, Camargo et al. 2004). Its score values (from 1 to 15) reflect the tolerance of each Iberian macroinvertebrate family to water pollution (organic pollution, mainly). The total BMWQ (t-BMWQ) is calculated by summing the individual scores of all families present in the sample, and the average BMWQ (a-BMWQ) is the quotient between the t-BMWQ value and the number of families.

The effect of each treatment (small, medium, large, mixture, slab, and Hess) on the different taxa densities (principal groups and families) and on the benthic metrics was assessed through a one-way ANOVA followed by a Tukey test (Zar 1984), after checking the homoscedasticity (Levene's test) (Levene 1960). When necessary, data were log-transformed. If necessary, the non parametric test Kruskal-Wallis was used (Zar 1984). A level of p<0.05 was chosen for ANOVA and Kruskal-Wallis tests. All statistical analyses were performed using SPSS 11.5 software.

RESULTS AND DISCUSSION

Among the main taxonomic groups (Amphipoda, Coleoptera, Diptera, Ephemeroptera, Hirudinea, Mollusca, Oligochaeta, Plecoptera, Trichoptera and Tricladida), only the mean densities of Coleoptera and Diptera were significantly different among treatments (ANOVA test, p<0.05) (Fig. 1). The densities of coleopterans in all artificial substrates were lower than those in Hess samples (Tukey test, p<0.05). The density of dipterans in the small treatment was higher than those in the mixture, slab, and Hess treatments (Tukey test, p<0.05), but not different from the rest of treatments (Tukey test, p>0.05).

There were significant differences among treatments for mean densities of Elmidae, Scirtidae, Chironomidae, Simuliidae, Glossosomatidae, Ceratopogonidae and Lumbriculidae (Table 1). However, the total densities of all individuals were not significantly different among treatments (ANOVA test, p>0.05). Neither diversity nor biotic indices differed between treatments (Table 2).

In general, coleopteran densities were lower in the artificial substrates than in the natural one. It might be argued that the 30-day colonization period was not long enough, as suggested by Barbour et al. (1999) who recommends a period of eight weeks for macroinvertebrate colonization. However, a period ranging from two to four weeks has been reported to allow periphyton colonization and development (Barbour et al. 1999), which is the main food source used directly or indirectly by several groups of invertebrates. Elmidae density (including adults and larvae of *Elmis* sp., *Esolus* sp., *Riolus* sp. and *Limnius volckmari*) was lower in the artificial substrates than in the natural one, excepting the mixture treatment (Fig. 2). A similar trend was found for Scirtidae (*Hydrocyphon* sp. larvae). Contrary to this, Fowler (2002) found a higher Elmidae density, caused by a quick colonization of artificial substrates baskets by drifting and crawling. The low Elmidae density found in the present work could be due to the artificial substrates design, as the lateral edge of the baked clay plate may have restricted the crawling and drifting of Elmidae (and other coleopterans). This problem could be

avoided removing the plate and using only stones inside the plastic basket (see design of Platts et al. 1983). However, that might favor colonization by the nearby fauna resulting in the high variability that natural communities exhibit in response to varying micro-conditions (Robson and Chester 1999, Beisel et al. 2000). Additionally, the inclusion of leaves inside the substrates could improve the colonization by scirtids (Daugherty and Juliano 2001).

In the case of dipterans, the highest density was found in the small substrate (Fig. 1). Chironomidae showed the highest density in that treatment (Fig. 2). As the stones of this substrate were very small (mean diameter of 1.9 cm), they did not exceed the top of the plate. This could have favored the accumulation of fine particulate organic matter (FPOM) and a velocity reduction inside the substrate in comparison with the other substrates with higher stones. These conditions might have favored several species of chironomids, as FPOM is their main source of food, and they prefer low-current microhabitats (Gregg and Rose 1985, Casey and Kendall 1996, Merrit and Cummins 1996, Shieh and Yang 1999, Tachet et al. 2000, Davis et al. 2001). Minshall and Minshall (1977) found a positive relationship between the amount of detritus and fine sediment and the Chironomidae density. Additionally, several authors have found that small pebbles and gravel tend to trap more fine particulate organic matter than coarse substrates do (Rabeni and Minshall 1977, Wise and Molles 1979). In the case of Simuliidae (Simulium sp.), no individuals were found in natural substrates; in contrast, the artificial substrates lodged high densities of this family, especially in the medium treatment (Fig. 2). The rocks of treatments with medium and large stones protruded from top of the plate, being more exposed to current, a condition which suits Simuliidae (Hynes 1970). Additionally, simulids have a high capacity to colonize free habitats, which can favor its presence in artificial substrates (Chutter 1968, Ulfstrad et al. 1974, Hemphill and Cooper 1983, Rosser and Pearson 1995).

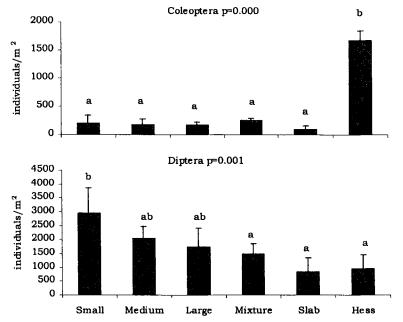


Figure 1. Mean densities and standard deviation for the main groups that significantly differed between treatments (ANOVA test, p<0.05). Different letters indicate significant difference between mean value of each treatment (Tukey test, p<0.05).

In the case of trichopterans, the Glossosomatidae (*Synagapetus* sp.) density in the slab treatment was lower than in the other treatments (Fig. 2), probably due to the absence of sand (<1 mm) in the former. Sand is required by Glossosomatidae larvae to construct their cases, therefore it is essential to complete its life cycle (Percival and Whitehead 1929). In addition, natural rocks support a more natural periphyton community than this in artificial materials, and this is an important food resource for this family (Rosser and Pearson 1995). The baked clay of the slab treatment had a flat surface that may have altered the natural composition of periphyton community, and this could have been less attractive for Glossosomatidae. Rosser and Pearson (1995) also found a higher number of Glossosomatidae (*Agapetus* sp.) on rocks than that on bricks.

Ceratopogonidae and Lumbriculidae families showed the highest densities in the Hess treatment. No individual of Lumbriculidae was found in any of the artificial substrates, and only medium and large treatments supported Ceratopogonidae individuals

Taxonomic group	Family	ANOVA		Kruskal-Wallis
		F	p	р
Ephemeropera	Baetidae	0.921	0.491	
	Caenidae			0.447
	Heptageniidae	2.281	0.093	
	Leptophlebiidae			0.229
	Ephemerellidae			0.560
Plecoptera	Nemouridae	1.633	0.205	
	Leuctridae	0.366	0.865	
	Perlidae			0.597
	Perlodidae			0.447
Trichoptera	Glossosomatidae	3.127	0.035*	
	Hydropsychidae	0.313	0.898	
	Hydroptilidae	0.010	0.000	0.688
	Limnephilidae	0.284	0.916	0.000
	Rhyacophilidae	0.401	0.010	0.842
	Philopotamidae	0.681	0.644	
	Sericostomatidae	0.001	0.011	0.589
	Lepidostomatidae			0.447
	Polycentropodidae	1.104	0.394	0.717
Coleoptera	Elmidae	4.737	0.007*	
	Scirtidae	5.159	0.005*	
	Hydraenidae	0.107	0.000	0.447
Diptera	Ceratopogonidae			0.019*
	Chironomidae	7.094	0.001*	0.017
	Simuliidae	2.867	0.047*	
	Stratiomyidae	2.013	0.128	
	Limoniidae	2.015	0.120	0.559
	Empididae	2.205	0.102	0.557
	Dixidae	2.202	0.102	0.447
	Psychodidae			0.407
Amphipoda	Gammaridae	2.003	0.130	0.407
Hirudinea	Glossiphoniidae	4.005	0.150	0.447
Mollusca	Bythinellidae	1.345	0.293	0.777
Monusca	Ancylidae	1.012	0.441	
	Lymnaeidae	0.365	0.865	
Odonata	Cordulegasteridae	0.505	0.005	0.447
Oligochaeta	Lumbriculidae			0.001*
Ongocnaeta	Naididae-Tubificidae	0.499	0.773	0.001
	Lumbricidae	0.477	0.775	0.447
Tricladida	Planariidae			0.204
Triciadida	r ianariidae			0.204

Table 1. Results of the ANOVA or Kruskal-Wallis tests for comparing densities (individuals/m²) of families of the various taxonomic groups. Asterisks show significant differences among treatments (p<0.05).

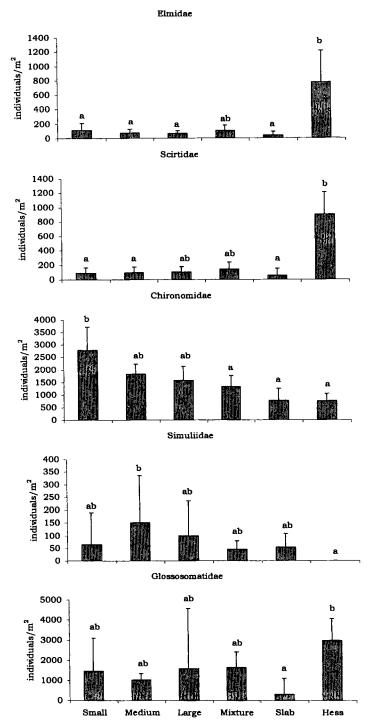


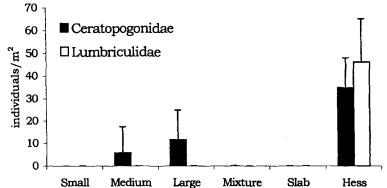
Figure 2. Mean densities and standard deviation for the families that significantly differed between treatments (ANOVA test, p<0.05). Different letters indicate significant difference between mean value of each treatment (Tukey test, p<0.05).

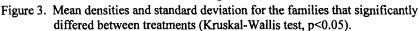
(Fig. 3). In contrast, other groups of Oligochaeta (Naididae-Tubificidae and Lumbricidae) were collected in all artificial substrates. A likely cause for this discrepancy is that Lumbriculidae and Ceratopogonidae drifts could be very scarce, reducing their potential to colonize new substrates.

Our artificial substrates recreated the natural community structure and quality in a precise way, as no significant differences were found for benthic metrics between treatments. Only taxa richness was close to being significantly affected by treatments (p=0.08) due to the scarce number of taxa found in the slab treatment. Our results agree with Fowler (2002) who found that the taxa richness and individual density were similar between artificial substrates constructed with materials of the river and the natural community. On the contrary, our results disagree with Casey and Kendall (1996, 1997) and McCabe and Gotelli (2003) who found higher densities and taxa richness in natural substrates than those in artificial ones. Moreover, Casey and Kendall (1996), who used a range of artificial substrate types, found that those constructed with natural materials supported the highest diversities, although they were lower than that of the natural community. Fowler (2002) and our own results suggest that the artificial substrates built with material from the stream bottom recreate the natural community structure. Natural materials can simulate the natural microhabitats and allow a more rapid development of fully productive surface organic layers than artificial materials (Ulfstrand 1968, Lake and Doeg 1985, Rosser and Pearson 1995). However, this type of sampling could overestimate the density of some Diptera (Chironomidae and Simuliidae) and underestimate the density of Coleoptera, especially Elmidae and Scirtidae.

Index	Туре	ANOVA	
Index	Type	F	р
Taxa richness	Diversity	2.408	0.080
EPT richness	Diversity	0.307	0.902
EPT density	Diversity	0.456	0.803
Camargo's diversity index (D)	Diversity	0.670	0.652
Camargo's dominance index (d)	Dominance	0.176	0.968
t-BMWQ	Biotic quality	1.276	0.319
a-BMWQ	Biotic quality	1.781	0.171

Table 2. Results of the ANOVA test for comparing benthic macroinvertebrate diversity, dominance and biotic indices.





ACKNOWLEDGMENTS

This study was funded by the research project (REN2001-1008) from Ministry of Science and Technology in Spain. Alcalá University provided logistical support to carry out this research. Álvaro Alonso was supported by a predoctoral grant from the Council of Castilla-La Mancha Community. Our sincere gratitude to Pilar for correcting the English text and to Marcos de la Puente for his friendly help with taxonomic identification.

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