



ARTIGO | ARTICLE

## Colonization of rocky and leaf pack substrates by benthic macroinvertebrates in a stream in Southeast Brazil

### Colonização do substrato rochoso e foliar por macroinvertebrados bentônicos em um riacho do Sudeste do Brasil

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#### ABSTRACT

A comparative analysis was conducted of the colonization by benthic macroinvertebrates of rocky and leaf pack substrates, both natural and artificial. This colonization was evaluated by season, with the objective of ascertaining the influence of rainfall on the rate of colonization. The total density of macroinvertebrates after 21 days of colonization was significantly greater in the dry than in the wet season. When the substrate types were compared, artificial leaf pack substrate presented the smallest density for both seasons. In the wet season, Chironomidae, Leptohiphidae, Hydropsychidae, Elmidae, immature stages of Trichoptera, and Hydroptilidae showed a more representative density. In the dry season, Chironomidae, Baetidae and Oligochaeta were the most abundant taxa. The artificial rocky substrate used in this experiment was the most appropriate, due to its resemblance with natural substrate conditions in terms of the maintenance of the structural integrity of the substrate throughout the experimental period. Successional and seasonal effects were of great relevance, playing an important role in the colonization process.

Key words: Artificial substrate. Colonization. Ecological succession. Experimental manipulation. Tropical streams.

#### RESUMO

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A colonização de rochas e pacotes de folhas, naturais e artificiais, por macroinvertebrados bentônicos foi analisada comparativamente. Esta colonização foi avaliada sazonalmente, com o objetivo de verificar a influência da pluviosidade sobre a taxa de colonização. A densidade total de macroinvertebrados após 21 dias de colonização foi significativamente maior na estação seca do que na chuvosa. Quando comparados os tipos de substrato, os menores valores de densidade foram observados para o substrato foliar artificial nas duas estações. Na estação chuvosa, Chironomidae, Leptohiphidae, Hydropsychidae, Elmidae, estágios imaturos de Trichoptera e Hydroptilidae apresentaram maior densidade. Na estação seca, Chironomidae, Baetidae e Oligochaeta foram os taxa mais abundantes. O substrato rochoso artificial utilizado neste experimento mostrou-se apropriado, devido à semelhança com o substrato natural quanto à estrutura e à integridade ao longo do período experimental. Efeitos sucessionais e sazonais foram de grande relevância, tendo um importante papel no processo de colonização.

Palavras-chave: Colonização. Manipulação experimental. Riacho tropical. Substrato artificial. Sucessão ecológica.

## INTRODUCTION

The experimental manipulation of natural communities can generate important contributions to the comprehension of the establishment, maintenance and persistence of communities (Hulberg & Oliver, 1979), and it can be used to evaluate the effects of various processes upon the structure of terrestrial, marine and freshwater ecosystems (Peckarsky & Penton, 1990). However, some caution should be exercised, since the structures utilized in the manipulation can modify the habitat, making it difficult to distinguish between the intended effects of the treatments realized and those effects due to non-intentional alterations in the habitat (Uieda, 1999). In manipulative experiments, it is important to test previously if the chosen substrate is adequate for the intended study and for the environment studied (Carvalho & Uieda, 2004; Ribeiro & Uieda, 2005), since various environmental factors may act in conjunction, structuring the communities (Miller, 1986). The substrate has been considered one of the most important factors, determining the distribution of invertebrates in the stream (Wise & Molles Jr., 1979; Allan, 1995).

Diverse types of artificial substrates have been utilized by various authors in experiments conducted in streams, such as clay tiles and sterilized rocks (Lamberti & Resh, 1985; Ribeiro & Uieda, 2005), galvanized wire baskets containing stones of different sizes (Wise & Molles Jr., 1979), aluminum trays filled with small stones (Freitas, 1998), metal trays with gravel and stones (Towsend & Hildrew, 1975), natural leaves in wire baskets (Walker, 1987; 1988; 1994),

and artificial leaves (Motta & Uieda, 2002). The most cited reasons for the utilization of artificial substrates are a reduction in the variability, effort and cost of sampling, and a decrease in the environmental impact (Lamberti & Resh, 1985; Freitas, 1998).

It is important to analyze if the structure of the artificial substrate provides the same conditions as the natural substrate, including shelter for organisms in periods of greater rainfall and, consequently, avoiding the dislodgement of invertebrates by virtue of the increase in discharge at this time. In this context, the objective of the present study was to perform a comparative analysis of the colonization process by benthic macroinvertebrates in natural and artificial substrates of two types, rocky and leaf pack. This experiment was performed during both the wet and dry seasons, with the objective of assessing the influence of rainfall on the colonization process.

## MATERIAL AND METHODS

The experiment was conducted in the Ribeirão da Quinta stream (23°06'47"S, 48°29'46"W), located in the municipality of Itatinga, São Paulo State, Southeast Brazil. This is a third-order stream, at an elevation of 743m AMSL, located on a cattle raising

farm, far from urban areas. At the experimental site, the stream had a well-preserved riverbank gallery forest on the left bank and abundant herbaceous vegetation on the right bank. The experiment was established in a run, characterized by a moderate current flow (0.15m/s), sandy-rocky bottom and small depth (30-40cm).

Four types of substrate were utilized in the experiment: artificial rocky, natural rocky, artificial leaf pack and natural leaf pack. The artificial rocky substrate was manufactured with cement and rocks, in a cobblestone pattern, and perforated by six small punctures; for the natural rocky substrate, nine stones, of the size predominant in the experimental area, were wrapped together with a 1 cm mesh galvanized screen in a such a way as to replicate the size and structure of the artificial substrate (Carvalho & Uieda, 2004). The leaf pack substrate was made with six leaves, plastic leaves for the artificial substrate and leaves sampled from the most common trees of the riverbank gallery forest for the natural one. The surfaces of the leaves were measured first (LI-COR area meter, model LI-3100) and were then also wrapped with the galvanized screen in such a way as to create a structure similar in size to the rocky substrate. The substrates were distributed randomly in the stream channel bed, attached to stakes using fishing line so as not to be dragged away.

The experiment runs during the wet season, from 21 November to 12 December, 2001, and the dry season, from 3 to 24 July, 2002. Samples were removed on the 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 13<sup>th</sup> and 21<sup>st</sup> days after the substrate installation, like in another experiment conducted in the same area (Carvalho & Uieda, 2004). At each date, three replications of each type of substrate were removed. The substrates were suspended carefully and packed in plastic flasks containing 70% alcohol.

### Data analysis

In the laboratory, the substrates were brushed and washed in the alcohol, which was emptied into three granulometric sieves (Granutest, mesh 1.00; 0.50 and 0.25mm). The macroinvertebrates

were sorted by the inspection of the sieves under a stereomicroscope. The animals were identified at the family level (Pennak, 1978; Lopretto & Tell, 1995; Merritt & Cummins, 1996), when possible, and counted for the determination of richness (number of taxa) and density (number of individuals per substrate area).

The density of macroinvertebrates at the 21<sup>st</sup> day of colonization was analyzed by One-Way ANOVA (Statsoft, 1996) to verify if there were significant differences between seasons and substrates. Density was log-transformed to comply with assumptions of parametric statistics. These data were also used to construct density-dominance curves of species, or curves of species importance, plotting the density of each taxa [ $\log_{10}(x+1)$ ] in the ordinate and the importance number of each taxa (rank), in decreasing order of density, in the abscissa (Brower & Zar, 1984).

The process of ecological succession was studied quantitatively by changes in the density of the eight taxa with the greatest density values throughout the process of colonization. For each taxa, the relative density on each day of sampling (1, 3, 7, 13, and 21 days of colonization, employed as the successional stages in this analysis) was determined and plotted on a graph against the successional stage (Brower & Zar, 1984).

## RESULTS

A seasonal variation was evident when analyzing the density of benthic macroinvertebrates sampled after 21 days of colonization, with the greatest richness found in the wet season (27 and 24 taxa, respectively in the wet and dry seasons), but twice the density in the dry season ( $F_{1,118} = 110.85$ ,  $p < 0.001$ ), although in both seasons the majority was represented by insects (Table 1).

With the wet season samples, there was a predominance of Chironomidae, Leptohiphidae, Hydropsychidae, Elmidae, Hydroptilidae, and immature stages of Trichoptera. With the dry season samples, there was a high prevalence of Chironomidae (more than 70%) followed, although in

Table 1. Density (D= number of individuals per m<sup>2</sup> of substrate area) of benthic macroinvertebrates taxa sampled at the 21<sup>st</sup> day of colonization on each substrate (NR- natural rocky, AR- artificial rocky, NL- natural leaf, AL- artificial leaf), during the wet (November-December 2001) and dry (July 2002) seasons of the Ribeirão da Quinta stream.

Taxa	Wet season				Dry season			
	NR	AR	NL	AL	NR	AR	NL	AL
Platyhelminthes-Turbellaria	16	32	40	66	0	0	8	0
Mollusca-Ancylidae	63	63	102	99	0	16	8	26
Annelida-Oligochaeta	587	349	112	141	889	365	831	602
Copepoda-Cyclopoida	0	0	0	0	0	0	25	0
Crustacea-Aeglidae	0	0	0	8	0	0	0	0
Acarina	32	32	0	0	16	0	9	0
Coleoptera-Elmidae	810	619	836	332	635	254	330	131
Coleoptera-Gyrinidae	0	0	0	8	0	0	0	0
Coleoptera-Psephenidae	0	0	0	8	0	0	0	0
Diptera-Ceratopogonidae	0	16	0	0	0	0	0	0
Diptera-Chironomidae	2540	2349	1120	622	12841	10540	10475	4919
Diptera-Empididae	16	48	0	17	95	32	41	30
Diptera-Psychodidae	0	16	8	0	0	16	0	0
Diptera-Simuliidae	143	48	16	0	127	190	16	8
Ephemeroptera-Baetidae	190	270	120	91	2317	1619	1363	522
Ephemeroptera-Caenidae	48	48	31	149	0	16	16	0
Ephemeroptera-Leptohyphidae	2095	1238	1227	655	238	190	190	172
Ephemeroptera-Leptophlebiidae	95	95	48	66	111	127	74	87
Hemiptera-Pleidae	0	0	0	17	0	0	0	0
Odonata-Aeshnidae	0	0	16	0	0	0	8	0
Odonata-Calopterygidae	0	0	24	8	32	32	8	8
Odonata-Coenagrionidae	16	16	0	8	16	0	8	15
Plecoptera-Gripopterygidae	0	32	0	0	63	32	16	15
Plecoptera-Perlidae	0	0	0	0	0	0	0	8
Trichoptera-Calamoceratidae	0	0	0	25	0	0	0	8
Trichoptera-Glossosomatidae	0	16	0	0	48	0	0	0
Trichoptera-Hydropsychidae	1317	1206	388	33	0	79	16	8
Trichoptera-Hydroptilidae	571	794	183	91	32	16	25	15
Trichoptera-immature stages	1032	841	154	41	48	63	16	8
Total density/substrate	9571	8128	4425	2485	17508	13587	13483	6582
% density/substrate/season	38.9	33.0	18.0	10.1	34.2	26.6	26.3	12.9

a low percentage, by Baetidae and Oligochaeta. Figure 1 facilitates the visualization of this seasonal variation in relative density for the seven most abundant taxa, some highly represented in one period with little or no representation in the other (Chironomidae was excluded from the figure because it was extremely abundant in the dry season;  $F_{1,22}=52.158$ ,  $p<0.001$ ). Leptohyphidae, Hydropsychidae, Elmidae, Trichoptera immature stages, and Hydroptilidae were significantly better represented in the wet season ( $F_{1,22}=20.157$ , 11.870, 5.380, 8.168, 15.895;  $p=0.0002$ ; 0.002; 0.03;

0.009; 0.0006; respectively). Otherwise, Baetidae and Oligochaeta were the most abundant taxa in the dry season ( $F_{1,22}=31.572$ , 10.617,  $p=0.00001$ , 0.004; respectively), after Chironomidae.

The density-dominance curves confirmed the greatest evenness of all substrates and, for the dry season curves, the high prevalence of Chironomidae (high density of rank 1 species at Figure 2). The presence was also evident of more rare species in the artificial substrates during the wet season. The results of the variance analysis, applied to the

species richness data, showed significant differences between wet and dry seasons ( $F_{1,22}=7.794$ ;  $p=0.01$ ), but not among different types of substrate ( $F_{3,8}=2.115$ ;  $p=0.18$ ).

Although the four substrates showed similar density-dominance curves and richness at the 21<sup>st</sup> day of colonization, a significant difference in density between substrates was verified ( $F_{3,8}=5.70$ ,  $p=0.023$  for the wet season data;  $F_{3,8}=7.80$ ,  $p=0.009$  for the dry season data), with the greatest difference observed when the natural rock and artificial leaf substrates were compared (Figure 3, Table 1).

Analyzing the relative density of the eight most representative taxa sampled in the rock and leaf pack substrates, over the successional process of colonization, during the wet and dry seasons (Figure 4), the density differences between substrates and seasons could be observed, although some taxa showed similar successional behavior when substrates or seasons were compared. Coleoptera-Elmidae and Trichoptera-Hydropsychidae started out at a low density but reached high density values at the end of the colonization process. On the other hand, Annelida-Oligochaeta and Diptera-Chironomidae increased early and fast in density but attained low

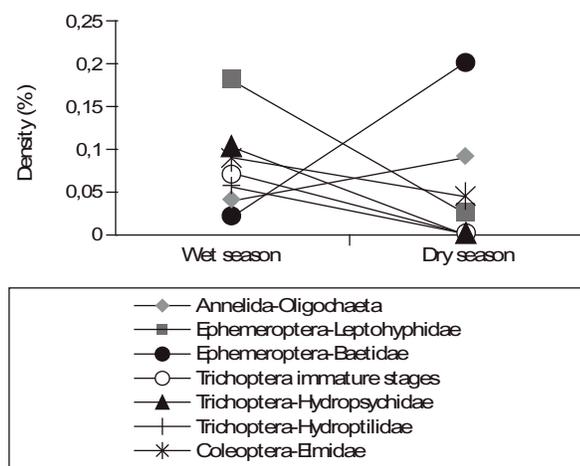


Figure 1. Relative density (%) of the seven most representative taxa, calculated in terms of the total community sampled during the wet and dry seasons (sum of the four substrate types), after 21 days of colonization.

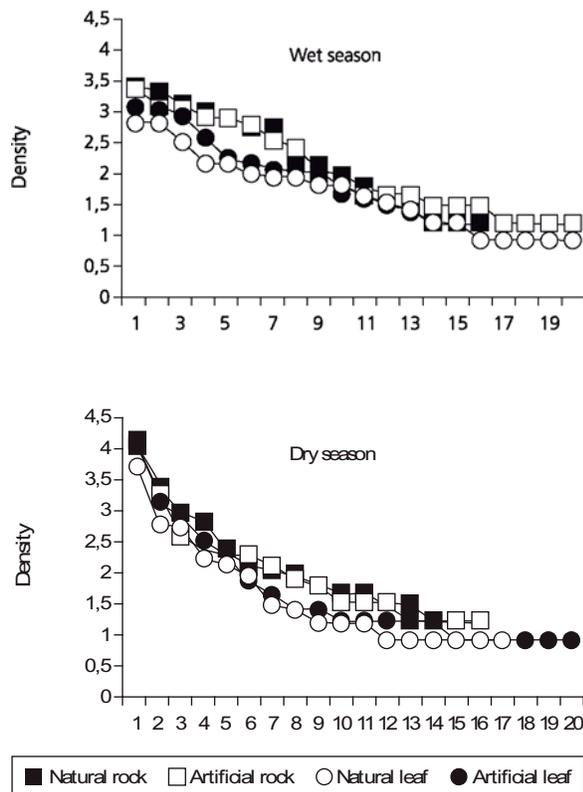


Figure 2. Dominance-density curves of taxa sampled at four substrate types: natural rock; artificial rock; natural leaf; artificial leaf [ $\log_{10}(x + 1)$  of the density at the 21<sup>st</sup> day of colonization], during the wet and dry seasons.

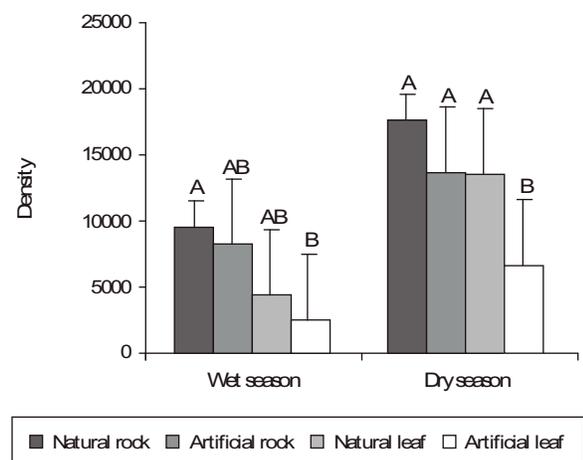


Figure 3. Density (number of individuals per m<sup>2</sup> of substrate area) of total macroinvertebrates sampled at the 21<sup>st</sup> day of colonization, during the wet and dry seasons, in the four substrate types: natural rock; artificial rock; natural leaf; artificial leaf (mean+1 standard deviation). Different letters indicate significantly different treatments (Tukey test).

density values by the end.

DISCUSSION

Of the diverse taxonomic groups that comprise the stream macroinvertebrate community, none has been studied more than the aquatic insects, a diverse 'both taxonomically and functionally (Hauer & Resh, 1996). Their great diversity in streams was also confirmed in the present work.

Studies, comparing the effects of the substrate on the colonization dynamic, showed that factors such as form, stability and particle size can be important (Freitas, 1998; Anjos & Takeda, 2005). In the present study the artificial substrate, mainly the rocky one, presented a colonization dynamic similar to that of natural substrate, suggesting that there was

a similarity in their physical substrate characteristics, that permitted a similar colonization process.

The densities in the natural and artificial rocky substrates were not significantly different, demonstrating that they are physically similar. Furthermore, the artificial substrate was shown to be adequate for the study of the local benthic community, facilitating substrate manipulation and the standardization of the sampling area. The vertical punctures made in the artificial rocky substrate probably allowed the movement of some organisms in the hyporheic zone. This zone has a diversified fauna (Dahm & Valett, 1996), that can move to and colonize the surface of the rocky substrate (Carvalho & Uieda, 2006). Otherwise, models, such as those employed by Townsend & Hildrew (1975) and Freitas

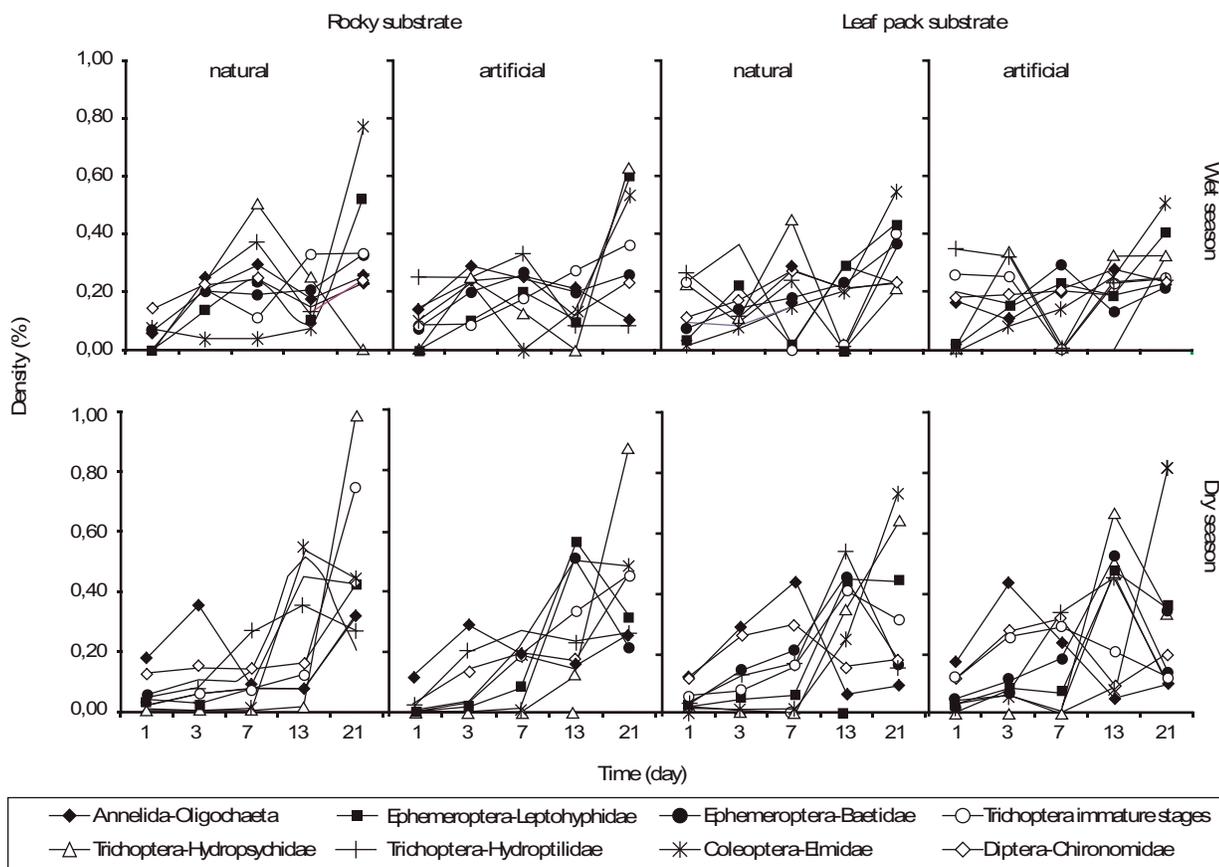


Figure 4. Relative density (%) of the eight most representative taxa sampled in the rock and leaf pack substrates, over the successional process of colonization, during the wet and dry seasons.

(1998), utilizing trays containing stones, can represent a barrier for vertical colonization.

The significant difference in density values found at the artificial leaf substrate is probably related to the constancy of its structure over the entire period of colonization, not suffering leaching and decomposition, which is common to the natural leaf substrate. Although a greater similarity between the artificial leaf substrate and the rocky substrate (natural and artificial) was expected, with both probably not changing their structure throughout the experiment, in fact the results showed greater similarity between the natural leaf substrate and the rocky ones. The expected outcome, though not achieved in this work, was found by Motta & Uieda (2002), using plastic leaves as artificial substrate in a manipulative stream experiment. Using a similar colonization period (28 days) these authors observed the establishment of a community similar to the natural and artificial leaf substrates, considering the use of artificial substrate advantageous, since it permitted the establishment of a similar community and homogeneity between samples.

The quantitative study of the process of succession, considering changes in density over a period of time, often shows an increase in density during the early stages of succession (Brower & Zar, 1984). In the successional process that occurred during the colonization of the substrates, Annelida and Diptera may be considered as fast and early colonizers, and Trichoptera and Coleoptera as late colonizers.

The results of density, richness and successional analysis reinforce the similarity among substrates and the strong seasonal effect on the community structure. The forces that shape community structure are those that determine which and how many species occur together, which are common and which are rare, and what are the interactions among them (Allan, 1995).

The high richness but low density during the wet season may be influenced by physical effects. In this season, the increase in rainfall can encourage a homogenization of substrates, allowing the

occurrence of more rare species in the absence of dominant groups. On the other hand, the dominance of Chironomidae and the less frequent occurrence of rare species in the dry season, can be associated with a reduced environmental effect, but with a strong effect from biotic interactions.

Biotic interactions can have a strong influence upon the community structure, enabling, under stable environmental conditions, the dominance of some species (Allan, 1995). Otherwise, a strong environmental pressure can defeat biotic interactions and define a more diverse community (Allan, 1995). In all probability, the analyzed community presented a species set that benefits from a strong environmental effect, such as in the wet season, and another species set whose structure is defined by biotic interactions, such as in the dry period. This seasonal change was characterized by an inversion of more dense groups, or even families of the same order of insects.

In summary, the utilization of artificial rocky substrate in colonization experiments appears to be more appropriate, due to its resemblance to natural substrate conditions, and to the maintenance of the structural integrity of the substrate throughout the experimental period. Successional and seasonal effects are of great relevance and need to be considered in colonization studies.

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