

## Colonization of benthic invertebrates in a stony river in Southern Brazil

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**Abstract:** The artificial samplers and naturals are important tools to demonstrate biological diversity as well the ecologic succession process which occurs in lotic environments. Thus, the objective of this research was to verify the colonization process on natural and artificial substrates in dry and rainy periods in Palmital River (União da Vitória, Paraná, Brazil) to verify the effects of the rainy season on the community and the use of artificial samplers in ecological studies with benthic community. The samplers ( $n=32$ ) were arranged in a longitudinal stretch (16 natural and 16 artificial samplers). Two replicas of each sampler were taken of at 2<sup>nd</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup>, 35<sup>th</sup> and 42<sup>nd</sup> days of colonization. The organisms were identified and were analyzed for faunal analyses. A total of 509 individuals were collected, with 371 being collected in the dry period and 138 in the rainy period. The community diversity of the benthic invertebrates was higher in natural substrate during the rainy period and during the dry period the community was more diverse in artificial substrate and there was a significant difference between the sample periods. Both samplers were efficient for samplings and colonization of the macroinvertebrate groups and the variation within rainfall patterns during the rainy season was determinant in the composition, richness and abundance of the organisms of study.

**Keywords:** Benthic macroinvertebrates; Colonization process; Artificial samplers; Precipitation; Seasonality.

### Colonização de invertebrados bentônicos em um rio pedregoso no Sul do Brasil

**Resumo:** Os amostradores artificiais e naturais são ferramentas importantes para demonstrar a diversidade biológica, bem como o processo de sucessão ecológica que ocorre em ambientes lóticos. Assim, o objetivo deste trabalho foi verificar o processo de colonização em substratos naturais e artificiais em períodos seco e chuvoso no Rio Palmital (União da Vitória, Paraná, Brasil). Para verificar os efeitos da estação chuvosa na comunidade e o uso de amostradores artificiais em estudos ecológicos com a comunidade bentônica. Os amostradores ( $n = 32$ ) foram dispostos em um trecho longitudinal (16 amostradores naturais e 16 artificiais). Duas réplicas de cada amostrador foram tomadas no 2°, 4°, 7°, 14°, 21°, 28°, 35° e 42° dias de colonização. Os organismos foram identificados e analisados para análises faunísticas. Um total de 509 indivíduos foram coletados, sendo 371 coletados no período seco e 138 no período chuvoso. A diversidade da comunidade dos invertebrados bentônicos foi maior em substrato natural durante o período chuvoso e durante o período seco a comunidade foi mais diversificada em substrato artificial e houve diferença significativa entre os períodos amostrais. Ambos os amostradores foram eficientes para amostragens e colonização dos grupos de macroinvertebrados e a variação dentro dos padrões de precipitação durante a estação chuvosa foi determinante na composição, riqueza e abundância dos organismos de estudo.

**Palavras-chave:** Macroinvertebrados bentônicos; Processo de colonização; Amostradores artificiais; Precipitação; Sazonalidade.

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## INTRODUÇÃO

Benthic macroinvertebrates fauna comprises a wide variety of organisms, with representatives of almost all phyla, such as Arthropoda (Insecta, Acarina, and Crustacea), Mollusca (Gastropoda and Bivalvia), Annelida (Oligochaeta and Hirudinea) among others, inhabiting at least part of their life at the bottom of aquatic ecosystems (CARVALHO; UIEDA, 2004; RIBEIRO; UIEDA, 2005; LIONELLO et al., 2011; DEMARS et al., 2012). They have huge ecological importance to many aquatic ecosystems, playing a key role in the dynamics of nutrients, a transformation of matter, and the flow of energy (HAUER; RESH, 2017).

Colonization is a series of processes that include population dispersal, establishment, and reproduction (WIRTH et al., 2008), and, depends on the individual's locomotion strategies, substrate texture and food availability brutal so on competition and predation relations with other biota components (MACKAY, 1992). The succession process begins when the organisms respond to physical priorities and colonize a new substrate (SUBIDA, 2008; BRACCIA et al., 2014). Brown & Brussock (1991) emphasize that despite the obvious differences in water flow, depth and slope between pools and riffles, other less obvious factors (like substrate composition) may also have pervasive influence on their suitability as habitats for macroinvertebrate species. In rivers, big stones as substrate guarantee high diversity, while litter keeps high diversity and abundance (CALDEIRA et al., 2013) and sand is, usually, associated to lower benthic invertebrate diversity (SHIMANO et al., 2010; LOSKOTOVÁ et al., 2019), so with the use of different substrates, different attributes can be evidenced in the benthic community.

The benthic macroinvertebrates fauna present in an ecosystem can be measured and characterized by colonization of patterned substrates (BICUDO; BICUDO, 2004; ESTEVES et al., 2011). Each type of substrate favors the

establishment of an invertebrate community, so it is interesting to understand how benthic invertebrates are established by exploring the substrate, in addition to visualizing the colonization process, due to the exact period of colonization beginning and the intervals in which each niche played by the invertebrate (MELO; FROELICH, 2001; RIBEIRO; UIEDA, 2005; SOUZA et al., 2006; SANTOS et al., 2016; CAMARGO et al., 2019). Thus, as the use of artificial samplers allows the standardization of the sample area and the period in which colonization and succession begin in the community, the objective of the work was to compare the colonization and the composition of the community of benthic macroinvertebrates using natural and artificial substrate in a stony river in southern Brazil.

## MATERIAL AND METHODS

### Study area

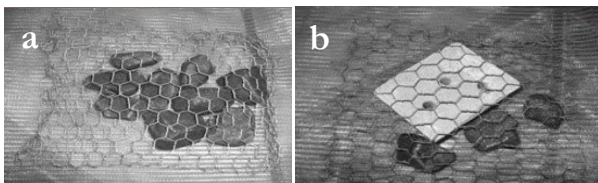
The experiment was carried out in a longitudinal section of the Palmital River, União da Vitória (Palmital do Meio locality) - Paraná, Brazil (26° 01' 55" S; 51° 08' 32" O; Figure 1). The characterization was carried out between the dry and rainy period from the analysis of the last ten years of historical records of precipitation (COPEL, 2011), thus, we define the dry period (of less precipitation in the year) from May to June 2011 and the rainy period (greater precipitation in the year) of November to December 2011.



**Figure 1.** Location the study in the Palmital River. **Fonte:** Google INC, 2020.

### Sampling

The natural substrate was formed by a set of nine river stones with approximately 2.8 cm of diameter; the stones were washed and bagged in wire netting (Figure 2a) with a total surface area similar to the artificial substrate. The artificial substrates made for this experiment consisted of blocks of cement (40%), fine sand (40%) and stones (20%), shaped like a rectangle (8.0 X 6.0 X 2.5 cm<sup>2</sup>) (Figure 2b). The artificial substrate was also bagged in wire netting to maintain the same experimental structure (CARVALHO; UIEDA, 2004).



**Figure 2.** (a) natural substrate. (b) artificial substrate.

In the study area 32 sample units were used, being 16 natural and 16 artificial, with a distance of 1.5 meters between the substrates, arranged sequentially (artificial/natural/artificial/natural). Two replicas of each substrate were taken from the river at 2<sup>nd</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup>, 35<sup>th</sup> and 42<sup>nd</sup> days of colonization. The substrates removed from the water were individualized and stored in plastic containers, identified and fixed in 10% formalin. In the laboratory, the sample units were washed and brushed in plastic trays. The material obtained from the washing was filtered using a 0.25 mm mesh sieve, sorted under an illuminated tray, and preserved in 70% ethanol. The benthic organisms were identified to the lowest possible taxonomic level using a stereoscopic microscope and specialized identification keys (ROLDAN, 1988; TRIVINHO-STRIXINO, 1995; MERRIT; CUMMINS, 1996; WIGGINS, 1996; MUGNAI et al., 2010).

### Data analysis

The T test ( $p < 0.05$ ) was applied to verify differences in abundance when compared to natural and artificial substrates between dry and rainy periods. For this analysis, in each sampling, the number of individuals in each replicate of the two types of substrates

was pooled separately, totalizing 8 samples of the natural substrate, and 8 samples of the artificial substrate for each period. Analyze of variance (Student's t test) were performed in Statistica 7.1 (Statsoft Inc., 2005).

Five descriptors based on the structure of the communities were calculated: (1) abundance; (2) richness; (3) diversity, calculated by the Shannon-Wiener; (4) equitability; and (5) Simpson's dominance. These attributes were calculated using the statistical package Past® (HAMMER et al., 2001).

The indicator species to the substrates and between the seasonal periods was defined by an Indicator Species Test (IndVal) (DUFRÊNE; LEGENDRE, 1997).

### RESULTS

In total, 509 individuals collected in the natural and artificial substrates in the two sampling periods. Of these, 371 were collected from the dry period, and 138 individuals were collected from the rainy period (Table 1).

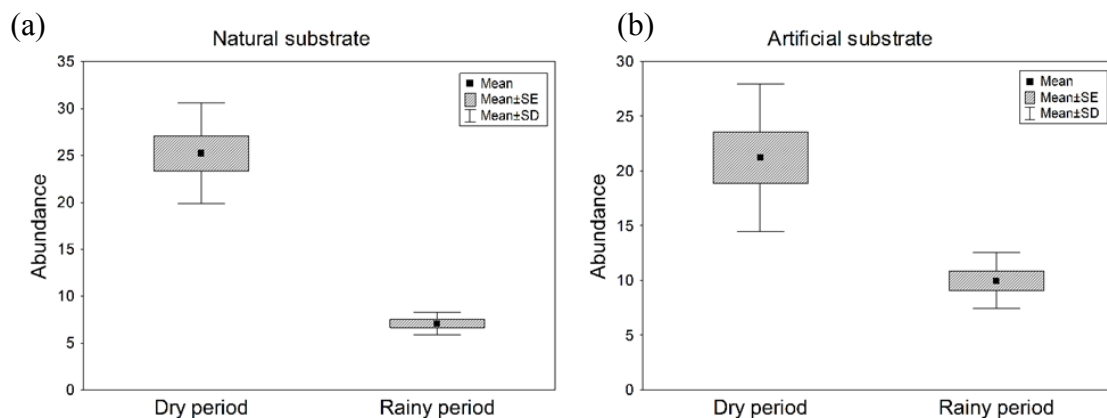
The most abundant taxa found in natural substrates were *Simulium* sp. (Diptera), members of the Orthoclaadiinae (Diptera), and *Camelobaetidius* sp. (Ephemeroptera); within the artificial substrate, the most abundant taxa were *Simulium* sp. (Diptera), *Farrodes* sp. (Ephemeroptera), and *Camelobaetidius* sp. (Ephemeroptera).

In the analysis of abundance throughout the colonization process, there significant difference in the natural substrate between periods (T test;  $p \leq 0.05$ ) (Figure 3a), and their significant difference in the artificial substrate between periods (T test;  $p \leq 0.05$ ) (Figure 3b).

The community diversity of the benthic invertebrates was higher in natural substrate during the rainy period; however, during the dry period the community was more diverse in artificial substrate. This same pattern was also observed with respect to species richness. The equitability observed did not vary between the samplers or the periods; however, the observed dominance was higher in the rainy period for the natural substrate, and in the dry period for the artificial substrate (Table 2).

**Table 1.** Taxonomic groups of invertebrates sampled in natural and artificial substrates over 42 days of colonization in the Palmital river during the seasonal periods.

| Taxonomic Groups<br>Substrates | Dry Period |         | Rainy Period |         |
|--------------------------------|------------|---------|--------------|---------|
|                                | Artificial | Natural | Artificial   | Natural |
| EPHEMEROPTERA                  |            |         |              |         |
| BAETIDAE                       |            |         |              |         |
| <i>Camelobaetidius</i> sp.     | 11         | 17      | 14           | 5       |
| <i>Tupiana</i> sp.             | 7          | 2       | 0            | 0       |
| <i>Cloeodes</i> sp.            | 4          | 4       | 3            | 11      |
| <i>Tricortopsis</i> sp.        | 0          | 0       | 8            | 7       |
| CAENIDAE                       |            |         |              |         |
| <i>Caenis</i> sp.              | 0          | 0       | 1            | 0       |
| LEPTOPHLEBIIDAE                |            |         |              |         |
| <i>Thraulodes</i> sp.          | 0          | 1       | 0            | 3       |
| <i>Farrodes</i> sp.            | 3          | 3       | 23           | 3       |
| <i>Tricorythopsis</i> sp.      | 0          | 0       | 8            | 7       |
| PLECOPTERA                     |            |         |              |         |
| GRIPOPTERYGIDAE                |            |         |              |         |
| <i>Paragripopteryx</i> sp.     | 11         | 7       | 2            | 1       |
| <i>Tupiperla</i> sp.           | 1          | 0       | 2            | 3       |
| <i>Gripopteryx</i> sp.         | 1          | 1       | 0            | 0       |
| PERLIDAE                       |            |         |              |         |
| <i>Macrogynoplax</i> sp.       | 0          | 1       | 0            | 0       |
| <i>Anacroneuria</i> sp.        | 0          | 0       | 1            | 0       |
| ODONATA                        |            |         |              |         |
| GOMPHIDAE                      |            |         |              |         |
| <i>Gomphoides</i> sp.          | 0          | 1       | 0            | 0       |
| HETERAGRIONIDAE                |            |         |              |         |
| <i>Heteragrion</i> sp.         | 1          | 0       | 0            | 0       |
| LIBELULIDAE                    |            |         |              |         |
| <i>Brechmorhoga</i> sp.        | 0          | 0       | 1            | 0       |
| COLEOPTERA                     |            |         |              |         |
| PSEPHENIDAE                    |            |         |              |         |
| <i>Psephenus</i> sp.           | 0          | 2       | 1            | 1       |
| DIPTERA                        |            |         |              |         |
| SIMULIIDAE                     |            |         |              |         |
| <i>Simulium</i> sp.            | 73         | 102     | 1            | 4       |
| CHIRONOMIDAE                   |            |         |              |         |
| Ortocladiinae (subfamily)      | 42         | 59      | 26           | 17      |
| Tanypodinae (subfamily)        | 5          | 3       | 0            | 0       |
| Chironominae (subfamily)       | 9          | 0       | 0            | 0       |
| TOTAL:                         | 168        | 203     | 83           | 55      |



**Figure 3.** Mean values of the abundance found in the natural and artificial substrates during the colonization between periods dry and rainy.

The community diversity of the benthic invertebrates was higher in natural substrate during the rainy period; however, during the dry period the community was more diverse in artificial substrate. This same pattern was also observed with respect to species richness. The equitability observed did not vary between the samplers or the periods; however, the observed dominance was higher in the rainy period for the natural substrate, and in the dry period for the artificial substrate (Table 2).

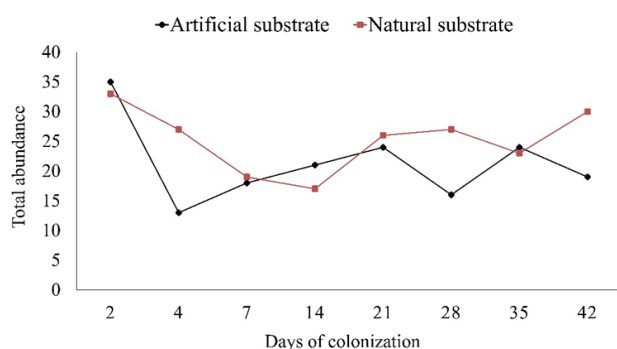
During the dry period, higher colonization indexes of natural substrate were: at the 2<sup>nd</sup> day with 33 individuals, 4<sup>th</sup> and 21<sup>st</sup> day with 27 individuals, and 42<sup>nd</sup> day with 30 individuals, the lowest index was at the 14<sup>th</sup> day of

colonization (n=17). On artificial substrate the higher colonization indexes were: at the 2<sup>nd</sup> day with 35 individuals, at the 21<sup>st</sup> and 35<sup>th</sup> day with 24 individuals, the lowest index was at the 4<sup>th</sup> day of colonization with 13 individuals (Figure 4).

During the rainy period, higher colonization indexes of natural substrate were: at the 4<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day with 8 individuals, the lowest index was at the 35<sup>th</sup> day of colonization (n=5). On artificial substrate the higher colonization indexes were: at the 2<sup>nd</sup> day with 15 individuals, at the 4<sup>th</sup>, 7<sup>th</sup> and 35<sup>th</sup> day with 10 individuals, and 42<sup>nd</sup> day with 12 individuals, the lowest index was at the 28<sup>th</sup> day of colonization with 7 individuals (Figure 5).

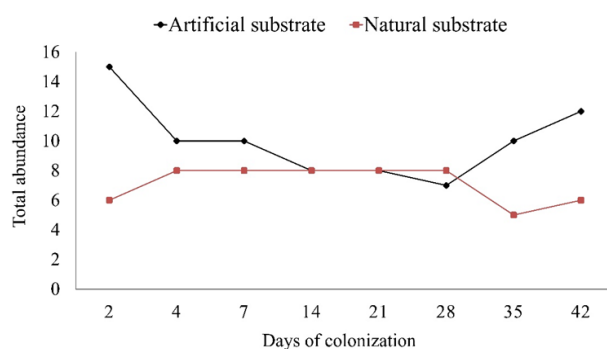
**Table 2.** Attributes of the communities: abundance (A), richness (S), diversity (H), equitability (J) and dominance (D) from the samples of the two studied seasonal period, Palmital river, União da Vitória - PR.

| Periods              | A   | S  | H'   | J'   | D    |
|----------------------|-----|----|------|------|------|
| <b>Dry period</b>    |     |    |      |      |      |
| Natural substrate    | 203 | 13 | 1.42 | 0.55 | 0.34 |
| Artificial substrate | 168 | 12 | 1.71 | 0.68 | 0.26 |
| <b>Rainy period</b>  |     |    |      |      |      |
| Natural substrate    | 55  | 11 | 2.03 | 0.84 | 0.16 |
| Artificial substrate | 83  | 13 | 1.88 | 0.74 | 0.20 |



**Figure 4.** Total number of individuals in each type of substrate per day of colonization in the dry period.

When assessing the capacity of specifying the species, according to the criteria of loyalty and specificity established by IndVal analysis, *Chironominae* (Diptera) and *Tupiarina* sp. (Ephemeroptera) were the most representative taxa of the artificial substrates in the dry period. On the other hand, *Tricorythopsis* sp. (Ephemeroptera) presented itself as a useful indicator species for the artificial and natural



**Figure 5.** Total number of individuals in each type of substrate per day of colonization in the rainy period.

substrates in the rainy period. *Simulium* sp. and *Paragripopteryx* sp. (Plecoptera), present in natural and artificial substrates in the dry period, can be used to indicate environments that do not present any changes in their integrity. These taxa presented environmental specificity to be used as indicators for this lotic environment.



## DISCUSSION

In the study, we verified a greater abundance and dominance of species in the colonization process in both samplers in the dry period. But we observed greater diversity and equitability of species in the rainy season. This occurs because in the rainy season there is greater availability of habitat and species that do not occur in dry periods, appear in the rainy season. As the drought increases on the site as the loss of critical stream habitat can provoke sudden changes in biodiversity and shifts in community structure (ASPIN et al., 2019).

The members of the Simuliidae family were pioneers and dominants, and occurred in both substrates in the dry period. This justifies its dominance during the beginning of the colonization process, which changes later because the wealth and the occurrence of the taxa decrease and then the colonizing species arrive, besides that, showed to be well adapted to the environment, which allows it to bear several abiotic factors (CARVALHO; UIEDA, 2004; CAMARGO et al., 2019)

Members of the subfamily Orthocladiinae (Diptera) demonstrated a high degree of dominance, as observed in other studies (BUENO et al., 2003; RIBEIRO; UIEDA, 2005), likely owing to their plasticity in establishing in lotic and lentic environments, as well their highly competitive abilities in both substrates, the diversity of this taxon was higher in the dry period, and this was likely due to the availability of nutrients when the sediment was removed (ABILIO et al., 2006)

Colonization of the substrates by Baetidae (Ephemeroptera), which occurred from the seventh day onward, led to a notable decrease in the abundance of Dipterans. In a process of succession, as the environment changes because of an increase in organic load in the substrate, the incoming taxa replace or exclude the pioneering organisms (BROWER, 1984). The pioneering organisms (Simuliidae) become disadvantaged in the competition for food and space, because late-arriving organisms, such as the Ephemeroptera, have greater capacity for locomotion, resulting in better food capturing capabilities (MARQUES et al., 1999, CARVALHO; UIEDA, 2004;

RAMIREZ et al., 2004; ASPIN et al., 2019).

Members of the order Diptera: families Chironomidae (KLEINE; STRIXINO, 2005; THOMAZI et al., 2008; PEREIRA et al., 2010) and Simuliidae (KIKUCHI; UIEDA, 2005) were representative of the benthic community in this river, as in other stony rivers. In general, lotic ecosystems with stones and gravel tend to have communities in which insects are the predominant component. This often includes Ephemeroptera and Plecoptera, which were respectively found here to be indicator species in the rainy and dry period. The presence of these indicator species is directly related to presence of stones, gravel, and water temperature (ARANHA; CARAMASCHI, 1997).

The strength of the water current during the rainy period was directly related to the diversity and abundance of the benthic community (THOMAZI et al., 2008). This is because it induces the dispersion of organisms and hampers the colonization process (LÓPES; COUTINHO, 2008) that occurs in the rainy period, when there is a decrease in the colonization rates of Diptera. These disorders cause decreases in abundance, mainly of the Chironomidae that are in the sediment (ZERLIN; HENRY, 2014).

Although the rate of succession was reduced in the rainy period, almost all the analyzed groups showed quick recovery, or even a high resistance, especially the ephemeral, which possess high levels of agility, hydrodynamic body forms, and tarsal nails that help fix them to the substrate (MELO; FROELICH, 2004; BAE; PARK, 2016).

The observed pattern of occurrence of Chironomidae in this study is similar to that verified by Aburaya and Callil (2007) at Itaúna Stream, a stony hydric body, where occurrence of the members of this taxon was noted to increase during the dry period. This is likely related to the removal of these organisms during the rainy period due to the water current (RIBEIRO; UIEDA, 2005) and to the increase in the size of substrate particles as, in spite of allowing stabilization in rainy periods, increased substrate particle size may affect the pre-existing community size as high degrees of friction with other smaller particles may

lead to a decrease in the amount of moss and periphyton covering present, thus reducing the availability of habitats (KIKUCHI; UIEDA, 2005).

The artificial substrate used was efficient in demonstrating diversity and richness to this river. Much like the natural substrate, the artificial substrate provides conditions to invertebrates that enable establishment, and provide food and shelter (HANSON et al., 2010; SHIMANO et al., 2010; SOUZA et al., 2011; SANTOS et al., 2016). Previous studies have shown a preference of invertebrates to settle on small stones (JOWETT, 2003; KORTE, 2010), and likewise, the species observed in the benthic community in these microhabitats seem to be gathered in a nested way when ordered with respect to the size of the stones (LOPES et al., 2009).

The process of colonization verified in this experiment showed similarity in richness between the two substrates during the sample period, following the same pattern as that found by Carvalho and Uieda (2004) in Ribeirão da Quinta. This demonstrates that the use of substrates for biological diversity analyses in lotic environments is efficient (CARVALHO; UIEDA, 2004; RIBEIRO; UIEDA, 2005; SILVEIRA; QUEIROZ, 2006; SOUZA et al., 2008, PEREIRA et al., 2010), and we encourage their use in diversity studies in this type of hydric body.

## CONCLUSIONS

The study performed here using natural and artificial substrates demonstrated that it is possible to analyze the benthic invertebrate community in the Palmital River, as well as the influence of various factors on this community. Variation within precipitation patterns during the rainy period and dry period was determined to be controlling factors of composition, richness, and abundance of the study organisms.

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