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Colonization of benthic invertebrates on artificial and natural substrate in a Neotropical lotic environment in Southern Brazil

Nilce Svarcz Jungles de Camargo[1], Atsler Luana Lehun[2*], Jonathan Rosa[2] and Ana Carolina de Deus Bueno-Krawczyk[1]

[1]Departamento de Ciências Biológicas, Universidade Estadual do Paraná, União da Vitória, Paraná, Brazil. [2]Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais, Núcleo de Pesquisas em Limnologia, Ictiologia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900. Maringá, Paraná, Brazil. * Author for correspondence. E-mail: atslerluana@gmail.com

ABSTRACT. The objective of this research was to validate the colonization process on natural and artificial substrates by benthic invertebrates in a tropical stream in South Brazil. The samples were performed in July and August-2012, and 32 samplers were used, being 16 natural and 16 artificial substrates. In each sample, two replicas were taken for each substrate at the 2th, 4th, 7th, 14th, 21th 28th, 35th and 42th days of colonization. The organisms were identified to the lowest possible taxonomic level. In both substrates 3,070 benthic invertebrates were detected, of which 1,753 individuals were collected on the natural substrate, and 1,317 on the artificial substrate. From the identified taxa 8.5% were not dominant (*Anacroneuria,* Orthocladiinae*, Tupiara, Smicridea, Baetodes, Tupiperla, Macrogynoplax, Gripopteryx, Cylloepus, Macrelmis, Microcylloepus, Hetaerina, Argia, Coryphaeschna, Atopsyche, Pomacea, Corydalus, Leptohyphes* and *Eccoptura*)*,* and 31.5% were dominant (Tanypodinae, Chironominae, and *Paragripopteryx*). The genus *Simulium* was very common, dominant and abundant, representing 65% of the collected individuals. No significant difference was found in the abundance and species composition between artificial and natural substrates. On the natural substrate, the higher colonization index was at the 35th day with 459 individuals, and the lowest was at the 14th day, with 87. On the artificial substrate the highest index was at the 42th day with 337 individuals, and the lowest was at the 4th day, with 85. Both natural and artificial substrates are efficient in characterizing the benthic community. In the evaluation of the ecological succession, it was not possible to observe a pattern that described the process, since the composition was nearly constant throughout the study period.

Keywords: Community structure; Diversity; Tropical stream; Limnology; *Simulium*.

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Introduction

Studies about diversity of the invertebrates in lotic environments integrate this ecological system as a whole, especially considering understanding the interaction among different taxonomic groups in aquatic communities. Benthic community has an important role in nutrients cycling and energy flux at the ecosystems since they participate on the organic matter fragmentation, decomposition and allow the ecological succession process (McCafferty, 1983; Bouchard Jr., 2004; Strixino & Trivinho-Strixino, 2006; Lisboa, Silva, & Petrucio, 2011; Bagatini, Delariva, & Higuti, 2012).

Based on the occupied habitat, aquatic invertebrates can be differentiated as planktonic that are associated with the water column or the surface film, and benthic, which are associated to the bottom and adjacent substrates (Weber, 1973).

The composition and spatial distribution of benthic aquatic invertebrates' fauna are related to several environmental factors, highlighting the water flow and the type of substrate. The water flow may act on the substrate nature, interfering in the structure of invertebrate communities (Whitton, 1975).

The succession process in environment begins when the organisms colonize a new substrate, in response to the physical priorities (Braccia, Eggert, & King, 2014). This succession dynamics in a peculiar area is initially recognized by the presence of less specialized taxa, also called pioneer organisms. They can change the site characteristics and determine the colonization by other individuals which extinguish their precursors (Carvalho & Uieda, 2004). Throughout the time this ecological succession process with the replacement of a community by another, involves not only a change in the composition of species, but also changes in biomass and environmental characteristics (Brower & Zar, 1984; Carvalho & Uieda, 2004).

The knowledge about the colonization process, richness and community structure of benthic invertebrates can be obtained by the use of substrates (Rosenberg & Resh, 1982; Ribeiro & Uieda, 2005). Several studies use natural and artificial substrates to characterize benthic fauna (Casey & Kendall, 1996; Santos, Bruno & Santos, 2016). Inorganic substrates have determinants in colonization as the size and surface, and the organic substrate has the feeding function, fixation and shelter (Rezende, 2007).

The artificial substrates are good to demonstrate primary succession process in the community and its structure (Carvalho & Uieda, 2004). The use of these samplers is efficient because it facilitates standardization of the sampling area and the time of the colonization. They are also important to evaluate the dynamics of aquatic invertebrates in relation to the environmental conditions they are exposed, such as the flow water of the stream and fluctuations of water level (Rodríguez, Becares, Soto, & Pacho, 1998). Taking into consideration the importance of the substrates, we aim to evaluate the effect of natural and artificial substrate on the richness, composition and abundance of the benthic invertebrate community in a neotropical stream.

Material and methods

Study area

This study was conducted in a longitudinal section of Papuã River, a tributary of the Iguaçu River (26ºS 07’04.1” and 51ºW 09’58.5”) at União da Vitória, Paraná, South of Brazil (Figure 1). Papuã River is 4 meters wide and about 58 cm deep. According to the river classification of Tundisi and Tundisi (2008) it is a small stream of 2nd to 5th order. The vegetation cover is present on both sides, composed mainly of Araucaria moist forest vegetation. It has an extensive rocky area, with rapids, shallow wells and sand near the banks, with little foliage and gravel, which permit to test succession process in an aquatic ecosystem with natural conditions.

All the replicas were taken in July and August 2012, during the low rainy period. The region climate is Cbf humid subtropical, according to the Köeppen classification. The average monthly rainfall was about 140 mm.

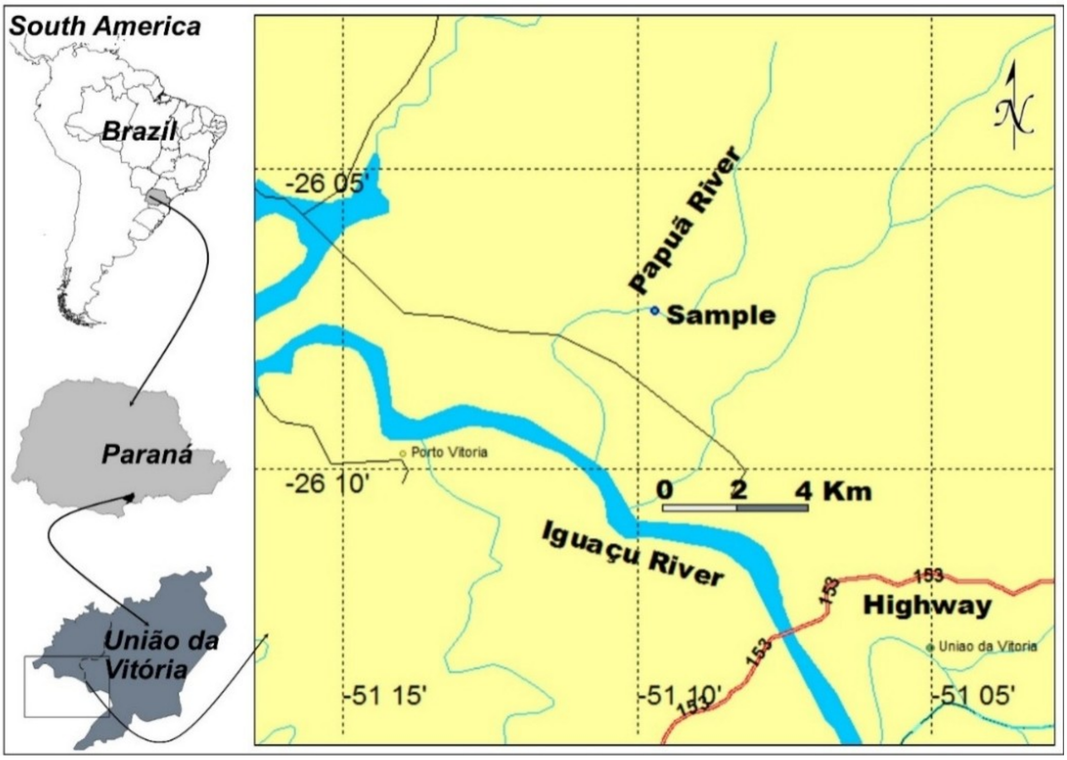

Figure 1. Location of the study area in the Papuã River, Paraná, Brazil.

Sampling

As the composition of the substrate of the Papuã River is predominantly rocks, sand and few leaves and gravel, we used artificial and natural substrates. Each artificial substrate consisted of a rectangular (8.0 × 6.0 × 2.5 cm) concrete block, with a total area of 210 cm^2, composed of cement (40%), fine sand (40%) and rocks (20%). Each natural substrate consisted of a set of nine river rocks, each about 2.8 cm in diameter, washed and dried on a wire mesh (1 cm mesh), with the total surface adapted to (1.5 × 1.2 cm) substrate (Carvalho & Uieda, 2004; Delonzek & Krawczyk, 2016).

In the study area 32 sample units were used, being 16 natural and 16 artificial, with a distance of 1.5 meter between the substrates. Two replicates of each type of substrate (artificial and natural) were removed from the stream at the 2^{th}, 4^{th}, 7^{th}, 14^{th}, 21^{th} 28^{th}, 35^{th} and 42^{th} days of colonization (Carvalho & Uieda, 2004). 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 illuminated tray and preserved in 70% ethanol.

The benthic organisms were identified to the lowest possible taxonomic level using stereoscopic microscope and specialized identification keys (Trivinho-Strixino & Strixino, 1995; Merrit & Cummins, 1996; Mugnai, Nessimian, & Baptista, 2010; Trivinho-Strixino, 2011). The identified organisms were deposited in the collection of the Zoology Laboratory of the *Universidade Estadual do Paraná*.

Data analysis

We analyzed data regarding the frequency of the individuals, abundance and dominance. We calculated the dominance on the dominance limit (LD), and with the resulting value compared to the frequency of each taxon. Frequencies smaller than the dominance limit (LD=4) is considered non dominant, while higher frequencies are said dominant (Silveira Neto, Nakano, Barbin, & Villa Nova, 1972). The relative frequency was calculated from each group present in relation to the total of individuals, considering that the taxa relative frequency is the result of the division of the number of individuals of a taxon by the total number of collected specimens (Dajoz, 1973).

The abundance of taxa was based on the calculation of the confidence interval on the number of the samples (Vieira, 1980). The following categories were assigned for the taxa: rare (<624 individuals); dispersed (⩾ 864 individuals); common (⩾ 891 individuals) and plentiful (⩾1,131 individuals). The T test ($p < 0.05$) was applied to verify differences in abundance when compared to natural and artificial substrates. 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.

Rarefaction curves were performed according to the number of individuals, in order to compare the species richness amongst the natural and artificial substrate at comparable levels of density (Gotelli & Colwell, 2001), using the program biodiversity professional version 2 (2018).

A Principal Coordinates Analysis (PCoA) was performed to evaluate the (dis)similarity of species composition between the substrates (natural and artificial). For the analysis, the axes were retained, according to the criteria of broken-stick method. The samples scores of the axes 1 and 2 were submitted to the Student's t test to assess the significance differences of species compositions between natural and artificial substrates. PCoA was carried out in R 3.4 software (R Development Core Team, 2016) using the vegan (Oksanen et al., 2019) and permute (Simpson, 2019) packages. Analyze of variance (Student's t test) were performed in Statistica 7.1 (Statsoft Inc., 2005).

Results

A total of 3,070 individuals were recorded on both substrates (natural and artificial). Both substrates used showed a significant number of individuals, 1,753 on natural substrate and 1,317 individuals on artificial substrate (Table 1). In the analysis of abundance throughout the colonization process, there was no significant difference between the two substrates (T test; $p = 0.20$), reinforcing the similarity between substrates (Figure 2).

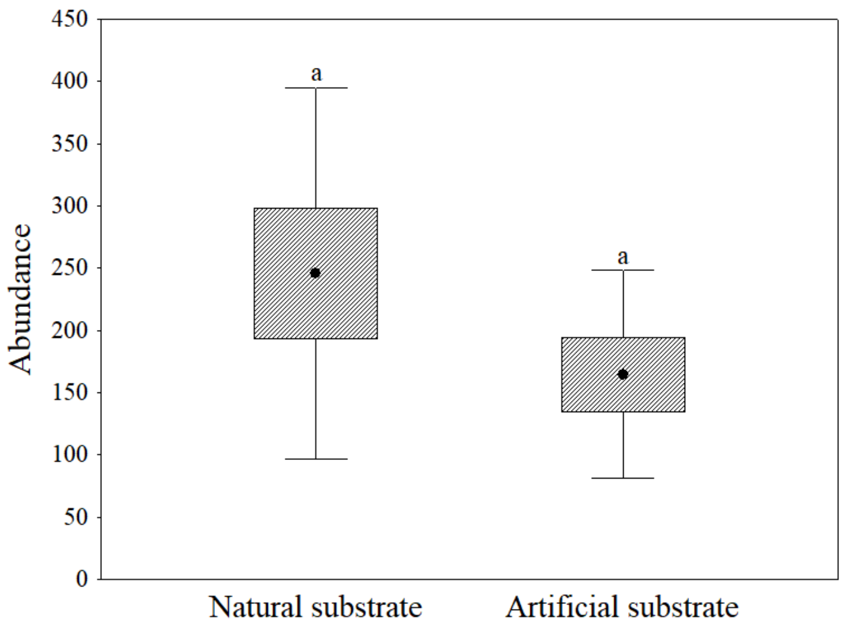

Figure 2. Mean values of the abundance found in the natural and artificial substrates during the colonization period. Bars represent standard error and same letters in columns indicate no statistical significance.

Table 1. Benthic invertebrates collected in artificial and natural substrates, over 42 days of colonization in Papuã River, União da Vitória - Paraná. (TF: individual frequency)

Taxa	Subfamily/genus	Natural substrate	TF (%)	Artificial substrate	TF (%)
Diptera					
Simuliidae	*Simulium*	1139	65	696	53
Chironomidae	Chironominae	135	7.7	156	11.8
	Tanypodinae	171	9.8	167	12.6
	Orthocladiinae	18	1	24	1.8
Plecoptera					
Gripopterygidae	*Paragripopteryx*	181	10.3	163	12.4
	Tupiperla	4	0.22	10	0.75
	Gripopteryx	2	0.15	3	0.22
Perlidae	*Anacroneuria*	55	3.13	50	3.8
	Macrogynoplax	1	0.05	6	0.45
	Eccoptura	9	0.5	5	0.4
Ephemeroptera					
Baetidae	*Baetodes*	9	0.5	6	0.45
	Tupiara	7	0.4	13	1
Tricorythidae	*Leptohyphes*	0	0	1	0.07
Coleoptera					
Elmidae	*Macrelmis*	1	0.05	0	0
	Cylloepus	1	0.05	2	0.15
	Microcylloepus	6	0.35	5	0.4
Odonata					
Calopterygidae	*Hetaerina*	1	0.05	1	0.07
Coenagrionidae	*Argia*	0	0	1	0.07
Aeshnidae	*Coryphaeschna*	0	0	1	0.07
Trichoptera					
Hydrobiosidae	*Atopsyche*	1	0.05	0	0
Hydropsychidae	*Smicridea*	12	0.7	5	0.4
Mollusca					
Ampularidae	*Pomacea*	0	0	1	0.07
Megaloptera					
Corydalidae	*Corydalus*	0	0	1	0.07

The rarefaction curves demonstrated that benthic invertebrate richness reached an asymptote in two type of substrates. The natural and artificial substrates showed similar numbers of individuals and taxa, but the highest richness was recorded in the artificial substrate (Figure 3).

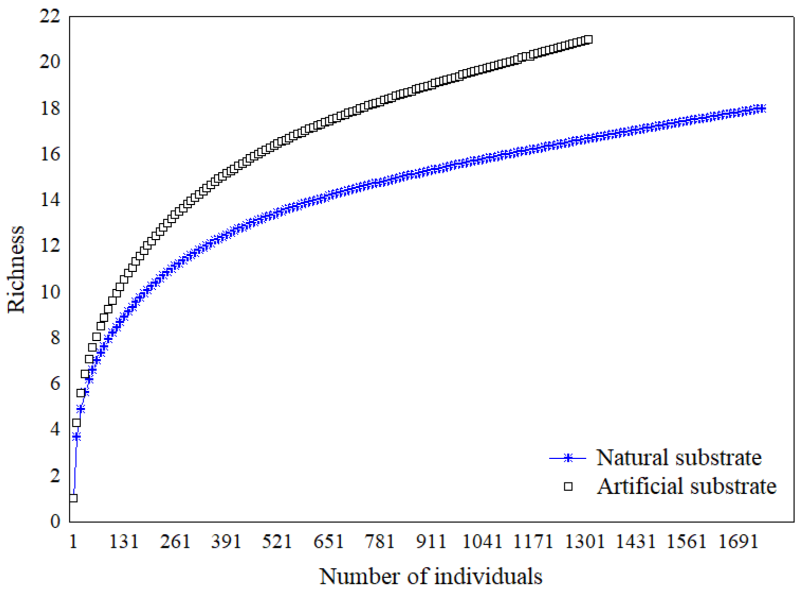

Figure 3. Rarefaction curves of benthic invertebrates in the sampling in substrate artificial and natural.

In both substrates the order Diptera was dominant. On natural substrate the species *Simulium* represented 65% and on artificial substrate 53%, Tanypodinae on natural substrate with 9.8% of representation and on artificial with 12.6%, Chironominae on natural 7.7% and on artificial 11.8%, followed by *Paragripopteryx* (Plecoptera) represented 10.3% on natural substrate and on artificial substrate 12.4%, the other taxa presented a number below than 4% in each substrate.

The genera *Pomacea*, *Corydalus*, *Argia*, *Coryphaeschna* and *Leptohyphes* were colonized only in artificial substrate, while *Macrelmis* and *Atopsyche* colonized the natural substrate. Comparing the artificial and natural substrates, we noticed that both presented a similar number of taxa over a period of 42 days of colonization, although the values in numbers of individuals were higher in natural substrate.

The colonization pattern on substrates were similar on both substrates, this suggests that diversity samplings in Papuã River can be done using these gears. The area showed 22 taxonomic groups less frequent and one very frequent (Table 2).

Table 2. Abundance and dominance of the taxa of benthic invertebrates in Papuã River, União da Vitória - Paraná.

Subfamily/Genus	Total	Abundance	Dominance	Frequency
Simulium	1835	Plentiful	Dominant	Very frequent
Chironominae	291	Rare	Dominant	Less frequent
Tanypodinae	338	Rare	Dominant	Less frequent
Orthocladiinae	42	Rare	Non dominant	Less frequent
Paragripopteryx	344	Rare	Dominant	Less frequent
Tupiperla	14	Rare	Non dominant	Less frequent
Gripopteryx	5	Rare	Non dominant	Less frequent
Anacroneuria	105	Rare	Non dominant	Less frequent
Macrogynoplax	7	Rare	Non dominant	Less frequent
Eccoptura	14	Rare	Non dominant	Less frequent
Baetodes	15	Rare	Non dominant	Less frequent
Tupiara	20	Rare	Non dominant	Less frequent
Leptohyphes	1	Rare	Non dominant	Less frequent
Macrelmis	1	Rare	Non dominant	Less frequent
Cylloepus	3	Rare	Non dominant	Less frequent
Microcylloepus	11	Rare	Non dominant	Less frequent
Hetaerina	2	Rare	Non dominant	Less frequent
Argia	1	Rare	Non dominant	Less frequent
Coryphaeschna	1	Rare	Non dominant	Less frequent
Atopsyche	1	Rare	Non dominant	Less frequent
Smicridea	17	Rare	Non dominant	Less frequent
Pomacea	1	Rare	Non dominant	Less frequent
Corydalus	1	Rare	Non dominant	Less frequent

The genera *Simulium* and *Paragripopteryx* and the ones from the subfamily Chironominae and Tanypodinae united had a frequency of 91.5% of all taxa sampled, consequently becoming the most recurrent within the taxa studied. While Orthocladiinae showed 1% and the other genera was less frequent.

The genus *Simulium* represented 60% of all collected individuals, presenting the highest frequency of individuals in the studied area, this genus is followed by: *Paragripopteryx* and Tanypodinae with 11%, and also having a significant representation Chironominae with 9.5%. These are in the families Simuliidae, Gripopterygidae and Chironomidae with the highest number of individuals.

The genus *Anacroneuria* had a frequency of 3.4% and Orthocladiinae 1.4%. The genera with frequency below 1% was: *Tupiara, Smicridea, Baetodes, Tupiperla, Macrogynoplax, Gripopteryx, Cylloepus, Macrelmis, Microcylloepus, Hetaerina, Argia, Coryphaeschna, Atopsyche, Pomacea, Corydalus, Leptohyphes* and the species *Eccopturaxanthenes*.

In this study, among the samples 8.5% less frequent, non-dominant and rare, are represented by nineteen taxa: *Anacroneuria,* Orthocladiinae, *Tupiara, Smicridea, Baetodes, Tupiperla, Macrogynoplax, Gripopteryx, Cylloepus, Macrelmis, Microcylloepus, Hetaerina, Argia, Coryphaeschna, Atopsyche, Pomacea, Corydalus, Leptohyphes* and *Eccopturaxanthenes*. Represented by two subfamilies: Tanypodinae and Chironominae and by one genus: *Paragripopteryx* 31.5% less frequent, dominant and rare.

Higher colonization indexes of natural substrate were: at the 2nd day with 353 individuals, at 28th day with 376, at 35th day with 459, and at the 42nd day with 335, the lowest index was at the 14th day of colonization (n=87). On artificial substrate the higher colonization indexes were: at the 2nd day with 167 individuals, at 7th day with 119, at 14th day with 155, at 28th with 139, at 35th with 228, and at the 42nd with 337, the lowest index was at the 4th day of colonization with 85 individuals (Figure 4).

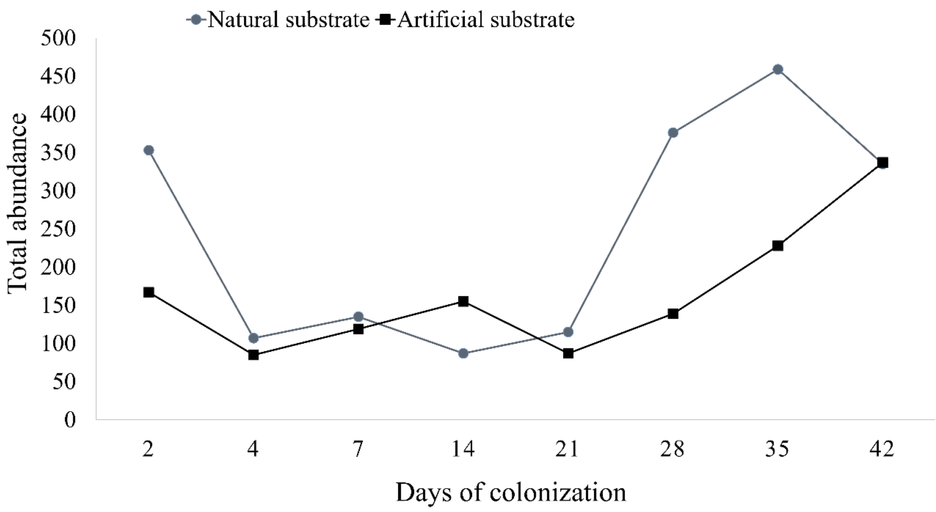

Figure 4. Total number of individuals in each type of substrate per day of colonization in Papuã River, União da Vitória - Paraná.

The results of PCoA did not show significant differences in the dissimilarity of species composition of the benthic invertebrates between the natural and artificial substrate ($p > 0.05$) (Figure 5). Thus, the composition was similar during the colonization process on both substrates.

Discussion

In our study there were few differences between abundances and total numbers of taxa on the natural and artificial substrates. Although there are no significant differences between both substrate types, some taxa were exclusive to the artificial substrate, such as Mollusca and Megaloptera. Probably this type of substrate provides a higher habitat heterogeneity, due to the interstitial spaces formed during the preparation of the substrate, favoring the colonization of exclusive taxa. Carvalho and Uieda (2004) also found no differences between both types of substrate (artificial and natural), but recorded exclusive taxa in the artificial substrate. According to Anjos and Takeda (2005), the higher structural complexity of the substrate favours the increase of species richness due to greater availability of resources and habitats.

A reduction in number of individuals was observed after the 4th day of colonization. Abiotic and biotic environmental conditions can reduce the abundance of aquatic invertebrates (Thomazi, Kiifer, Ferreira

Junior, & Sá, 2008), for example, predation by fish is a factor controlling the abundance of invertebrates in streams (Lovell, Fletcher, Cooper, & McArthur, 2017). However, in this study, no change was observed in the abiotic conditions of the environment during the colonization process, but the biotic variables were not measured in the field and may be a possible factor responsible for the reduction of organisms.

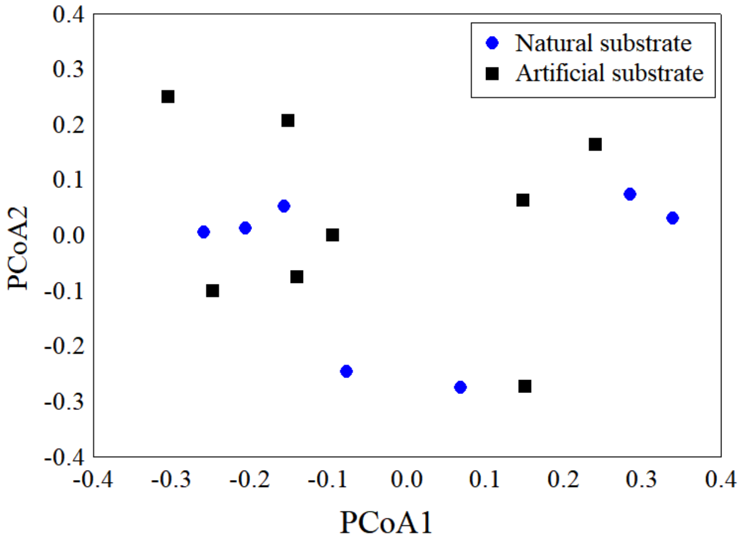

Figure 5. Composition's Dissimilarity of the benthic invertebrate's community in natural and artificial substrates.

Among the several groups found, Diptera is the most widely distributed and frequently the most abundant group in freshwater environments (Armitage, Cranston, & Pinder, 1995). In this study, it was the most representative group, being present throughout the sampling period and presenting no difference by substrate preference.

There was a prevalence of three families with a more significant number of individuals registered: Simuliidae and Chironomidae (Diptera), Gripopterygidae (Plecoptera) which can also be corroborated in studies such as Carvalho and Uieda (2004), Ribeiro and Uieda (2005), Pereira et al. (2010), where abiotic factors influence the ecological structuring of aquatic biotopes, determining the occurrence and distribution of organisms and their structuring.

Lisboa et al. (2011) and Rocha, Medeiros and Andrade (2012) described that Chironomidae was distributed in subfamilies Chironominae, Tanypodinae and Orthocladiinae, and these taxa were the most constant and diverse in community, especially because these families have distinct morpho-behavioral characteristics (Silva, Pauleto, Talamoni, & Ruiz, 2009; Trivinho-Strixino & Strixino, 1995). Furthermore, there are reports indicating that Chironomidae tends to increase during dry season (Aburaya & Callil, 2007), which happened in this study, probably as a result of the preservation of the riparian forest around the river and, consequently, great food availability.

Chironomidae are the most representative of aquatic insects as a result of the range of habitat occupancy; the use of various resources, which confers adaptive strategies to colonize different types of micro-habitats by different genera of the family (Trivinho-Strixino & Sonada, 2006). This wide range of occurrence in terms of Chironomidae can be explained by their diversified eating habits (Armitage et al., 1995). In general, the Chironomidae family does not require any special substrate for its development (Entrekin, Wallace, & Eggert, 2007).

The genus *Simulium* showed to be well adapted to the environment, which allows it to bear several abiotic factors. According to Ribeiro and Uieda (2005) rainfall was the factor that influenced the most the structuring of benthic community. As in the rainy season, the rocky substrate and the Simuliidae, are subjected to the direct action of the current, these taxa probably migrate and seek shelter on the vegetable substrate. Most species are found on the underside of rocks, branches, or leaves living in fast-moving waters (Statzner, 1981).

The abundance of aquatic invertebrate species in riparian areas are extensively discussed in the literature (Uieda & Gajardo, 1996; Nemeth, 1998; Kikuchi & Uieda, 2005). Thus, two hypotheses may explain the great colonization in current habitats: 1) the abundance of such in that environment is due to the plenty amount of oxygen and food; 2) faster colonization in this environment occurs due to the drift process, where the aquatic organisms detach themselves from the substrate to which they were attached and attach themselves to a new underwater substrate below (Merritt & Cummins, 1996).

According to Strixino and Trivinho-Strixino (2006), many benthic invertebrates are detritivores, feeding on organic matter produced in the water column. The accumulation of debris gives the aquatic invertebrates a great amount of food resources and when present in satisfactory quantity it can become attractive to benthic fauna (Rosenberg & Resh, 1982; Merrit & Cummins, 1996).

Thus, benthic invertebrates present great importance in these ecosystems, serving as a link between the basal resources (debris and algae) and fish and crustaceans, participating in the energy flow and the cycling of the nutrients (Bueno, Bond-Buckup, & Ferreira, 2003; Carvalho & Uieda, 2004). Therefore, knowledge on the community structure is a fundamental step in understanding the agreement of interspecific relations and the ecosystem as a whole (Molozzi, Salas, Callisto, & Marques, 2013), being important in the evaluation and conservation of freshwater systems (Jiang, Xiong, Xie, & Chen, 2011).

Conclusion

We concluded that the use of natural and artificial substrates is efficient for the sampling and colonization of the groups of aquatic invertebrates in tropical streams, being an important tool for ecological studies. In addition, artificial substrates provide a high heterogeneity of habitat, which is able of supporting species composition similar to the natural substrate. In the evaluation of the ecological succession, it was not possible to observe a pattern that described the process, since the composition was practically constant throughout the study period.

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