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COLONIZATION BY MACROINVERTEBRATES ON AN ARTIFICIAL SUBSTRATE AND COMPARISON WITH A NATURAL SUBSTRATE IN A FLOODPLAIN: A SHORT TIME STUDY IN THE RAINY SEASON

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ABSTRACT

Understanding how the process of colonization by taxa in different types of habitat takes place is necessary to preserve their diversity. The aim of this study was to analyze the process of colonization by macroinvertebrates on an artificial substrate with morphology similar to that of the roots of aquatic macrophytes *Ricciocarpus natans* and *Spirodela intermedia*, which were present in the environment investigated. The hypothesis is that the values of total density on the artificial substrate will show a tendency similar to that of a macrophyte stand, but with a different taxonomic composition. The differences in taxa density and composition associated with the artificial substrate and macrophytes were significant. The total density and richness on the artificial substrate did not follow an upward curve with a significant increase in the number of individuals during the experiment, probably due to the heavy rainfall observed. The variation in the density values in both substrates shows that the macroinvertebrate community was maintained through an "emigration" and "immigration" dynamic. Our hypothesis was confirmed only in relation to the taxonomic composition. The colonization of the artificial substrate by macroinvertebrates might have been more efficient had the environment not received frequent rains, which affected the physical stability of the macroinvertebrate community. Therefore, intrinsic sampling site characteristics may have attenuated the effect of the flood pulse on the macroinvertebrate communities, since only oscillations in the density values of the taxa were observed, rather than their exclusion.

Keywords: ecological succession; hydrological disturbance; phytofauna; *Ricciocarpus natans*; *Spirodela intermedia*.

INTRODUCTION

The organization and spatial distribution patterns of natural communities appear to be largely affected by interactions between environmental perturbations and biological processes (Sousa 1984). Nature management, as well as the preservation of biological diversity, depends on knowledge of the strength that effectively maintains the biodiversity of ecosystems (Tilman 1994). One of the main challenges in ecology is to understand how and when physical habitat heterogeneity controls the structure and function of biotic communities (Cardinale *et al.* 2002). Therefore, it is important to understand how taxa colonization occurs in different types of habitats. Organisms with low mobility can be considered true colonizers because they tend to effectively remain in the environment until some factor promotes their removal or departure (Mackay 1992). In inland water environments, colonization may occur quickly due to oviposition by winged adult terrestrial insects which inhabit water during part of their life cycle (egg and larvae phases), but their activity and the number of eggs deposited *per* substrate area unit are rarely reported (Mackay 1992).

Macroinvertebrate colonization at a determined site depends not only on the individual's locomotion strategies, substrate texture and food availability, but also on competition and predation relations with other biota components (Mackay 1992). An important issue when examining macroinvertebrate colonization in a particular habitat is how to define the beginning of the process of colonization of the site precisely. According to Ribeiro & Uieda (2005), experiments using artificial substrates can be useful because they enable the standardization of the sampling area and the initial time of colonization. However, it is important to check whether the structure of the artificial substrate presents the same characteristics as the natural substrate (Carvalho *et al.* 2008).

In addition to the above-mentioned factors, colonization success is also related to the taxa dispersal potential and abundance in adjacent areas (Brederveld *et al.* 2011). In the present study, artificial substrate samplers were surrounded by aquatic macrophytes *Spirodela intermedia* and *Ricciocarpus natans*. Both macrophytes can thus be a source of potential colonizers of the artificial substrate in the present study. Fontanarrosa *et al.* (2013) and Albertoni & Palma-Silva (2006) observed a rich and abundant macroinvertebrate community associated with small and medium-size floating macrophytes, including *R. natans* and *S. intermedia*, respectively. *Spirodela intermedia* has fasciculate roots up to 3.0 cm long (Pott & Cervi 1999) and *R. natans* has a total length from 1.0 to 1.5 cm (Pott & Pott 2000). According to these authors, *S. intermedia* is commonly found in conjunction with other species of floating aquatic plants, for example, *R. natans*.

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). However, when the environment is disturbed in different frequencies and intensities, distinct models of ecological succession may be inducted (Maltchick & Florin 2002). The sequence of stages including alterations in taxa composition and in the environment must be determined when ecological succession is examined. In the climax phase, equilibrium is attained when the percentage of introduction of new taxa into the community is equal to the percentage of disappearance of others and, thus, the richness becomes approximately constant (Mackay 1992). However, generalizations on an ideal timeframe of efficient colonization must be avoided, because colonization patterns differ with substrate types (Booth *et al.* 2013) and temporal scales (Thomazi *et al.* 2008).

Many macroinvertebrates use aquatic macrophytes as food resource sites (Lodge 1991, Newman 1991, Natchtrieb *et al.* 2011, Watson & Barmuta 2011). Since artificial substrates do not supply any source of energy to herbivorous macroinvertebrates, the working hypothesis is that some taxa with herbivore feeding habits will not be observed on the artificial substrate, but that the total macroinvertebrates density on the artificial substrate will show an increasing trend over time, with values similar to those observed on the natural substrate (macrophytes). This hypothesis is preliminarily supported by Barker *et al.* (2014). In their comparative study of macroinvertebrates associated with *Eichhornia crassipes* and an artificial substrate analog to its roots, they noted the existence of significant differences in fauna structure between the two substrates in analysis. One of the major causes of this was the nutritional role played by macrophyte roots to some taxa.

The goals of our study were: 1) to analyze the macroinvertebrates ecological succession through colonization process of an artificial substrate morphologically similar to the roots of aquatic macrophytes *R. natans* and *S. intermedia* (natural substrates) found in the environment, and 2) to compare the density, richness and composition of macroinvertebrates on artificial and natural substrates.

This research aims to contribute to the scientific knowledge of the dynamic of macroinvertebrate colonization on an artificial substrate, as well as the association of macroinvertebrates with small-size aquatic macrophytes. Most papers in the literature refer to the relationships of macroinvertebrates with large-size macrophytes. The analysis of the fauna structure of macroinvertebrates that inhabit aquatic plants with reduced root systems may shed light on the role that these macrophytes play in the aquatic environment as food sources and alimentation and shelter sites in the development of the fauna and on their eventual contribution to the spatial heterogeneity of habitat.

MATERIAL AND METHODS

Study area

The area selected for study is a floodplain marginal to the Guareí River located on a stretch within a preserved forest area (Angatuba Ecological Station, southeast São Paulo State, Brazil). Three sites located in the riparian forest were sampled: S1 (23°25’30.7"S, 48°19’21.4"W), S2 (23°25’26.4"S, 48°19’21.6"W) and S3 (23°25’27.8"S 48°19’23.2"W). The study area (Middle Guareí River) is part of the Jurumirim reservoir watershed that is located about 45 km far from the confluence zone with the reservoir (Figure 1). The predominant aquatic vegetation within the study area was dominated by the floating macrophytes *R. natans* and *S. intermedia*.

Experimental design and sampling frequency

Circular artificial substrate samplers were made of polyester carpet (Figure 2) in the same dimensions of the aquatic macrophyte sampling area (a circular sieve 250 µm mesh screen of area 0.035 m^2) and fixed to pieces of Styrofoam with hot melt adhesive and pins. Eighteen samplers were fixed to the margin and incubated in mixed stands of *R. natans* and *S. intermedia* at the three different temporary floodplain sites. One substrate sampler and a sample of macroinvertebrate fauna associated with the macrophyte stand were collected at each of the three sites at six different intervals of time from December 13th, 2012 to January 10th, 2013 (1st, 3rd, 7th, 14th, 21st and 28th days after the start of the exposition). Aquatic macrophyte sampling was inserted under the macrophyte beds and pulled from the water with the macroinvertebrates associated with macrophytes retained in the sieve.

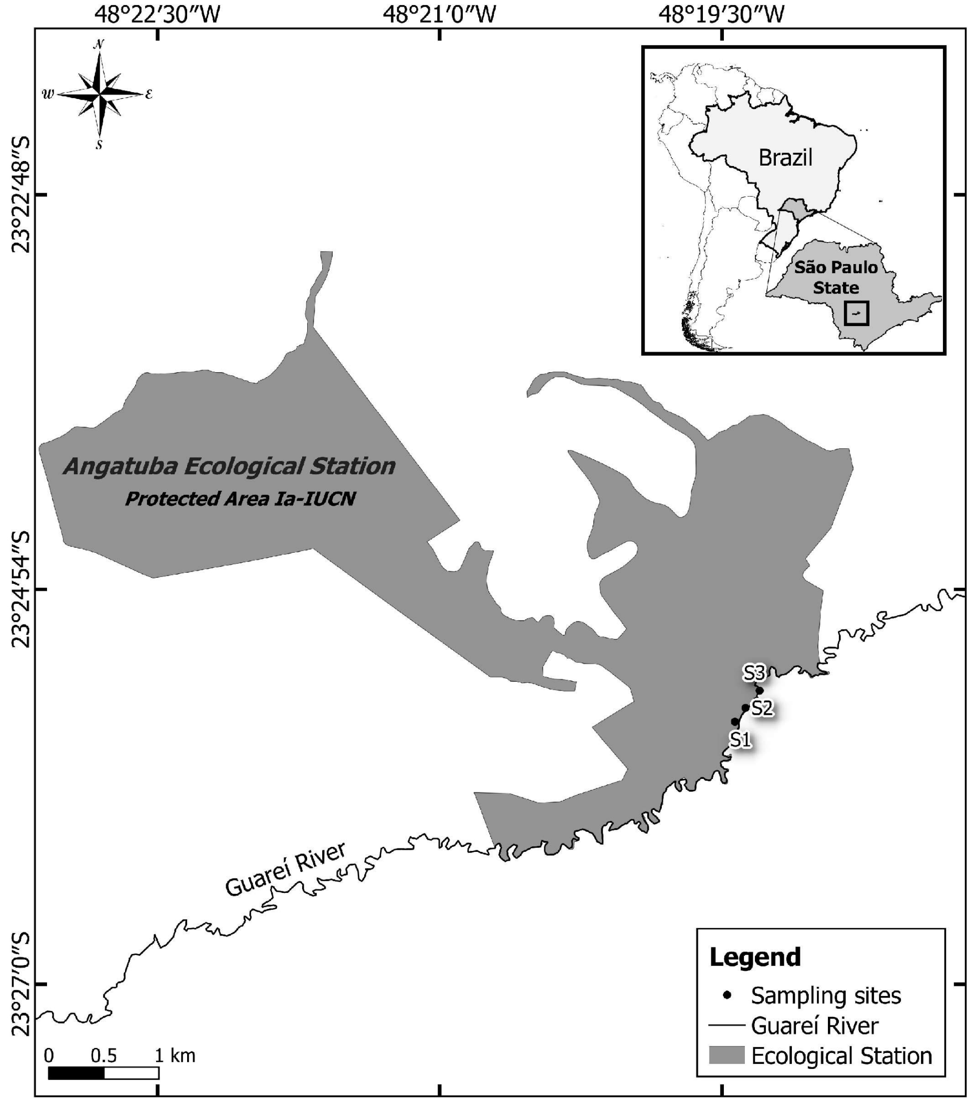

Figure 1. Study area at the Angatuba Ecological Station and the three sampling sites on a stretch of Middle Guareí River, in the State of São Paulo, Brazil.

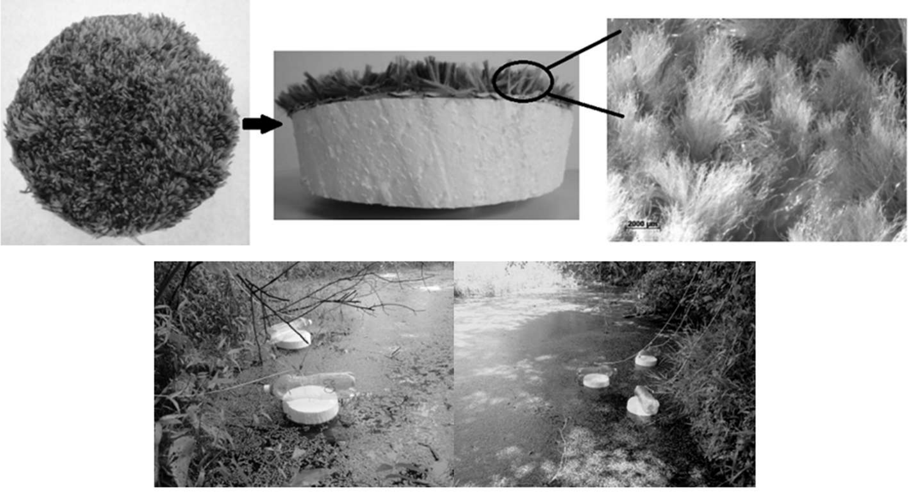

Figure 2. Artificial substrate sampler made of polyester carpet and experimental design with the position of artificial substrate samplers on the margin of Guareí River, State of São Paulo, Brazil.

Abiotic and biotic variables

Daily precipitation data during the study period were provided by the meteorological station at Angatuba Ecological Station. For each sampling site, the following variables (with the exception of water transparency) were determined at the water surface within the macrophyte stands: water temperature (mercury thermometer), dissolved oxygen (Winkler's method, modified by Golterman *et al.* 1978), water pH and electrical conductivity (corrected to 25°C, according to Golterman *et al.* 1978), suspended matter (gravimetry, following Teixeira & Kutner 1962), water transparency (Secchi disk), and total concentration of photosynthetic pigments (Golterman *et al.* 1978). Exclusively the water temperature and transparency were measured *in situ*. The water samples for the determination of suspend matter and photosynthetic pigments were collected by a plastic bottle and filtered in the laboratory by filters Millipore AP40. The total suspend matter concentration was obtained through the difference of the initial and final weight of the filters (after oven drying). The photosynthetic pigments were extracted in acetone (90%) for about 12 hours and the concentration determined with a spectrophometer, according to Golterman *et al.* (1978).

On each sampling day, three artificial substrate samplers were collected and removed from their Styrofoam support, individually transferred to plastic containers and fixed with a 70% alcohol solution. In the laboratory, the artificial substrates were carefully washed with water over a tray and examined under a stereoscopic microscope to ascertain the complete removal of macroinvertebrates. Next, the tray content was filtered with a 250-µm mesh screen and the macroinvertebrates were sorted out under a stereoscopic microscope and identified through reference to specialized literature (Domínguez & Fernandez 2009, Mugnai *et al.* 2010). The same procedure was applied to fauna samples associated with macrophytes collected at the three sites. After fauna removal, the plants were dried in an oven (60°C) to determine their biomass (grams of dry weight).

Data analysis

The mean daily colonization rate (x) of macroinvertebrates on the artificial substrate, expressed in terms of abundance (daily variation rate of individual abundance) and richness (daily variation rate of taxa richness) in the successive sampling intervals was estimated through simple derivate calculation, which function was macroinvertebrate abundance and richness over time using $x_A = (A_2 - A_{1)}/(t_2 - t_1)$, where A = abundance and t = time, and $x_R = (R_2 - R_1)/(t_2 - t_1)$, where R = richness and t = time. The Simpson dominance index was also computed (Odum & Barret 2007).

Statistically significant differences (p < 0.05) between sampling days in biotic and abiotic variables were analyzed with variance analysis using software Sigma Plot 11. Multiple data comparisons were carried out through Tukey's test. "One-way" ANOVA was used when the data were normally distributed and the Kruskal-Wallis test when the data did not present normal distribution.

The ecological attributes and macroinvertebrate densities in the macrophyte stand and the artificial substrate were compared with the "t" Student test when the data were normally distributed and the Mann-Whitney test when the data showed a non-normal distribution.

To test the similarity in macroinvertebrate density and composition (presence or absence) between the macrophyte stands and the artificial substrates on successive sampling days, Non-Metric Multidimensional Scaling analysis (NMDS) was done using the Bray-Curtis dissimilarity coefficient. The main taxa responsible for groupings shaped by the NMDS were identified by the SIMPER analysis and the differences in macroinvertebrate density and composition by the ANOSIM analysis. All analyses were carried out through program Primer v6 (Clarke & Gorley 2006) using square root-transformed data (Clarke & Warwick 2001). All the statistical tests (except in NMDS, SIMPER, and ANOSIM analyses) were carried out using (log (x + 1)) transformed data, except for pH values.

RESULTS

Abiotic variables

After three successive days of intense rainfall, the Guareí River overflowed on December 16, 2012 and inundated the lateral plain at Angatuba Ecological Station, affecting the sites where the artificial substrate samplers were exposed (Figure 3).

The highest water temperatures were recorded during the second half of the experimental period (Figure 4a). Dissolved oxygen and percent saturation presented low values on all the sampling days, especially from the 14th day on (Figure 4b). Evident variation in water pH was observed at the beginning of the experiment without any apparent difference from the 14th day of sampling onwards (Figure 4c). An opposite pattern was observed for water electrical conductivity, which presented a great variation during the study period, with the highest value on the 21st day (Figure 4d). A predominance of inorganic fraction in suspended matter was observed throughout the study, except on the 28th day. In relation to the total suspended matter, the highest concentrations were recorded on the 1st, 7th and 14th days (Figure 4e). Concerning water transparency, the lowest value was obtained on the 7th day (Figure 4f). Significant differences were pointed out only for water electrical conductivity (1st day ≠ 21st day; p = 0.004) and temperature (7th day ≠ 1st day; p < 0.001).

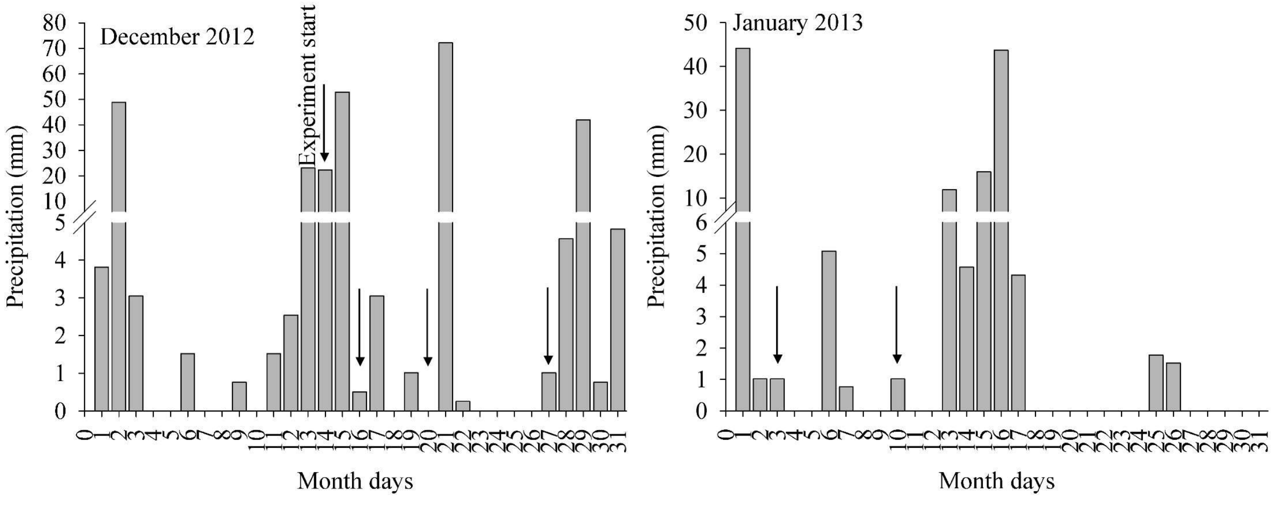

Figure 3. Daily precipitation (mm) in December 2012 and January 2013 at Angatuba Ecological Station, State of São Paulo, Brazil. Note the difference in scales. The arrows indicate the sampling days.

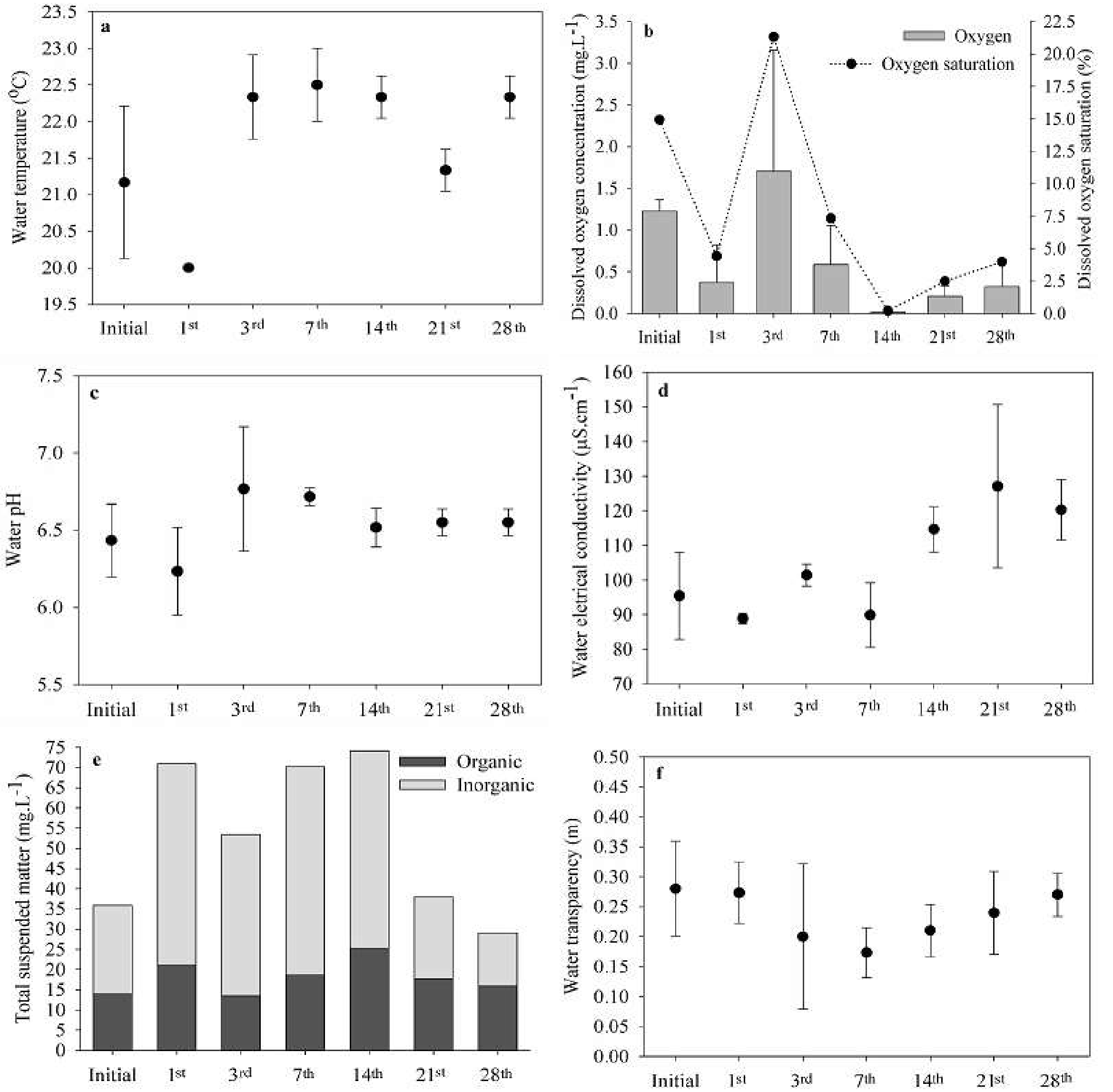

Figure 4. Mean ± SD (N = 3) of water temperature (a), dissolved oxygen and percent saturation (b), pH (c), electrical conductivity (d), organic and inorganic suspended matter (e) and transparency (f) on the plain lateral to the Guareí River on successive sampling days from December 2012 to January 2013. Note the difference in scales.

Biotic variables

Total pigments from water samples varied during the experiment and the highest value was observed on the 3rd day (13.8 µg.L^{-1}) (Figure 5a). No evident variation in macrophyte biomass was observed during the experiment (Figure 5b).

The density of macroinvertebrates associated with macrophytes (Figure 6a) was higher than that on the artificial substrate (Figure 6b). A rising density curve was observed on the artificial substrate from the 1st to the 7th days; the abundance decreased on the 14th day and increased only from the 28th day (Figure 6b). Comparing the fauna associated with the artificial substrate and macrophytes, differences were pointed out for total density (t-test $p<0.001$), total richness (t-test $p=0.028$) and macroinvertebrate density, except for Nematoda, Acari and Odonata. On the temporal scale, differences were obtained only for the total density of the fauna associated with the macrophytes (1st day≠ from all other successive sampling days; $p < 0.001$) and for Nematoda (1st day≠7th, 14th, 21st and 28th days; $p < 0.001$). In relation to the density in the artificial substrate, Insecta (1st day≠3rd and 7th days; $p = 0.033$), Turbellaria (1st day≠ 21st day; $p = 0.028$), Acari (1st day≠14th, 21st and 28th days; $p = 0.008$), Crustacea

(1st day≠ 7th day; p = 0.035) and Diptera (1st day≠ 3rd, 7th, 14th and 28th days; p = 0.005) differed during the experiment.

The mean daily variation rate in the number of macroinvertebrate organisms colonizing the artificial substrate showed an abundance peak on the 3rd day (144 ind.day^{-1}) and negative values on the 14th and 21st days (Figure 6c). An evident increase in the taxa richness daily rate was recorded on the 1st day of the experiment, decreasing significantly on the 3rd and 7th days, and was very low during the remainder of the experiment (Figure 6d).

The mean taxonomic richness of fauna associated with macrophytes varied from 18 to 21 taxa (Figure 6e) and on the artificial substrate it attained a maximum value on the 3rd and 28th days (15 taxa) and was approximately constant during the remainder of the experiment (Figure 6f).

During the study period, Crustacea, Insecta and Annelida were the most numerous groups of invertebrates on the artificial substrate (Figure 7a), but high abundance in the macrophytes was limited to the first two (Figure 7b).

The density of aquatic insects on the artificial substrate (Figure 8a) was lower than in the macrophyte stands throughout the experiment (Figure 8b). In both substrates, Diptera predominated during the study period and Ephemeroptera was the least numerous Insecta order (Figure 8a and b).

Diptera, Oligochaeta, Ostracoda, and Amphipoda presented the highest relative abundances on the artificial substrate (Figure 9a). The range of abundance of Diptera varied from 19.0% (1st day) to 42.7% (3rd day), Oligochaeta from 16.7% (7th day) to 35.0% (1st day), Ostracoda from 1.3% (3rd day) to 22.1% (28th day), and Amphipoda from 7.8% (28th day) to 27.0% (1st day) (Figure 9a). The most abundant taxa in the macrophyte stand during all the sampling period were Diptera and Amphipoda (Figure 9b). On the 3rd day, Diptera corresponded to 44.6% of the total abundance of macroinvertebrates, while Amphipoda attained 49% at the beginning of the sampling (Figure 9b).

The variation in the dominance index (range: 0.2-0.3) of the macroinvertebrate community associated with the artificial substrate and macrophytes during the experiment period were similar.

NMDS analysis showed that the density (Figure 10a) and composition (Figure 10b) of macroinvertebrates associated with the artificial substrate and the macrophytes differed on the spatial scale. However, no significant difference occurred on the temporal scale for either composition in the artificial substrate (ANOSIM: p = 0.558, global R = -0.021) or density in the macrophytes (ANOSIM: p = 0.628, global R = -0.046).

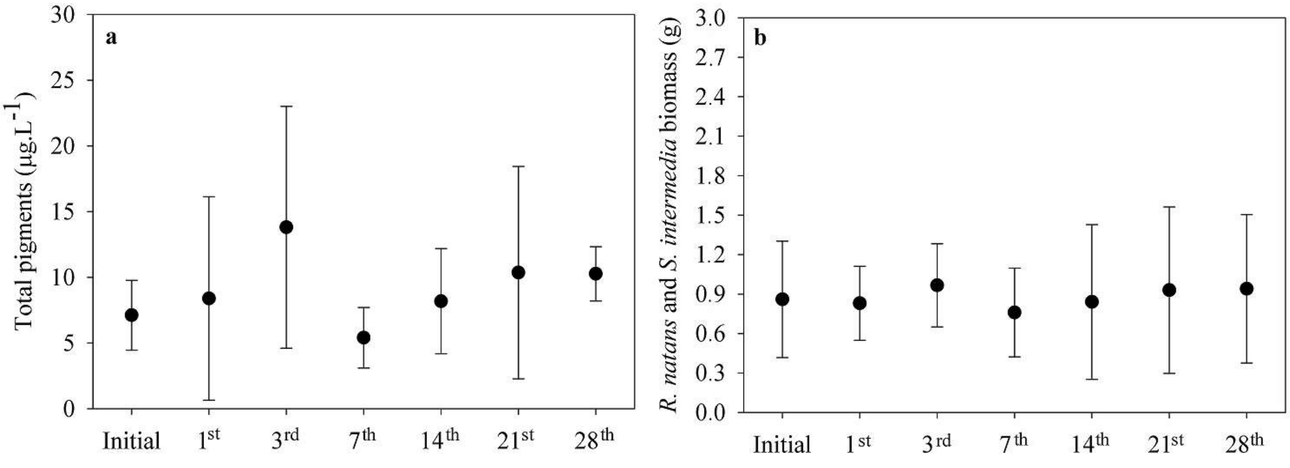

Figure 5. Mean ± SD (N = 3) of total pigments from water samples (a) and dry biomass of *Ricciocarpus natans* and *Spirodela intermedia* (b) on the plain lateral to the Guareí River, State of São Paulo, Brazil, on successive sampling days from December 2012 to January 2013.

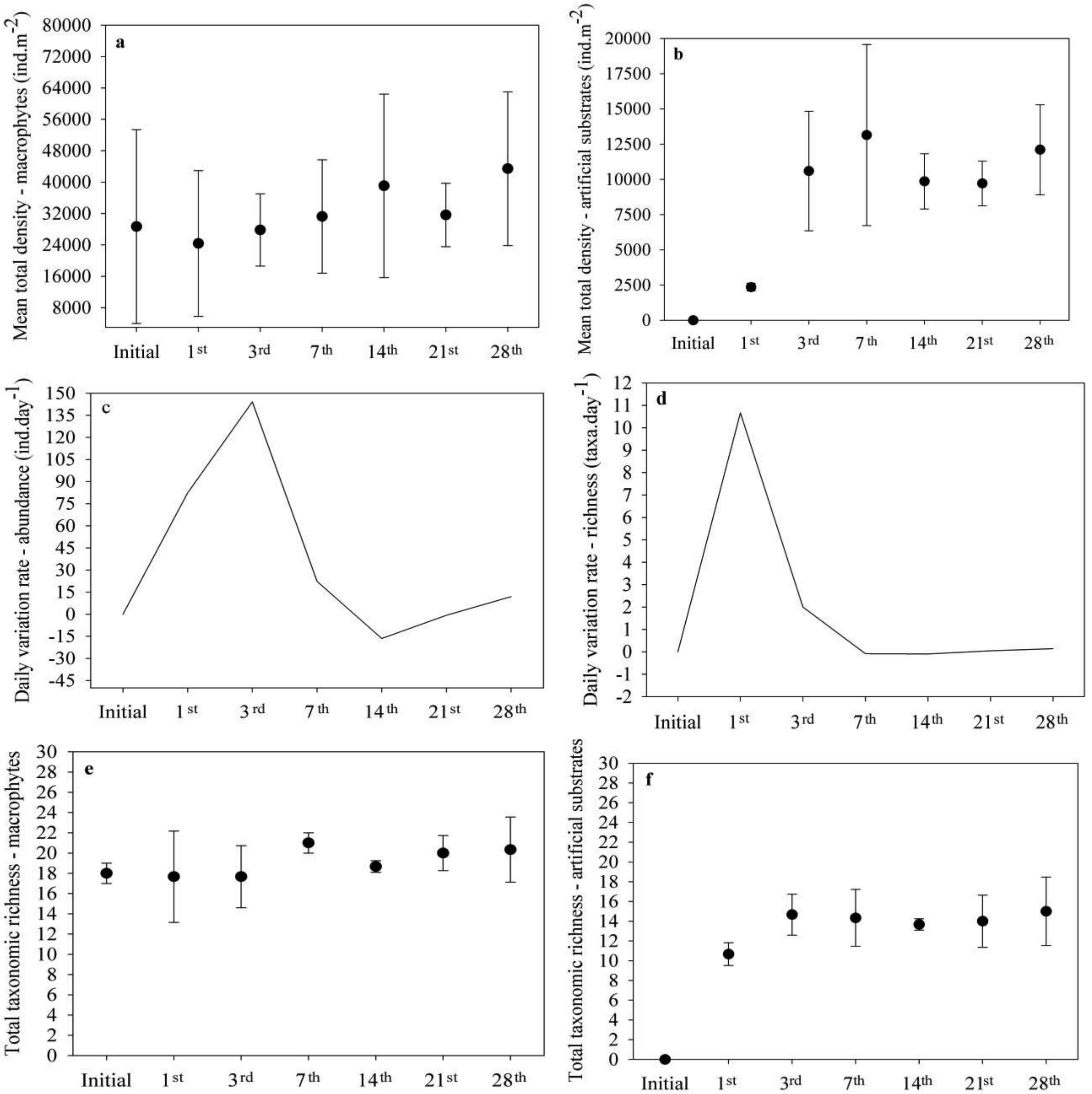

Figure 6. Mean ± SD (N = 3) of the total density of macroinvertebrates associated with macrophytes (ind.m^{-2}) (a) and on the artificial substrate (b), daily rates of variation of abundance (ind.day^{-1}) (c), and richness of macroinvertebrates (taxa.day^{-1}) colonizing the artificial substrate (d); total taxonomic richness of fauna associated with macrophytes (e) and on the artificial substrate (f) on the plain lateral to the Guareí River, State of São Paulo, Brazil, in successive sampling days from December 2012 to January 2013. Note the difference in scales.

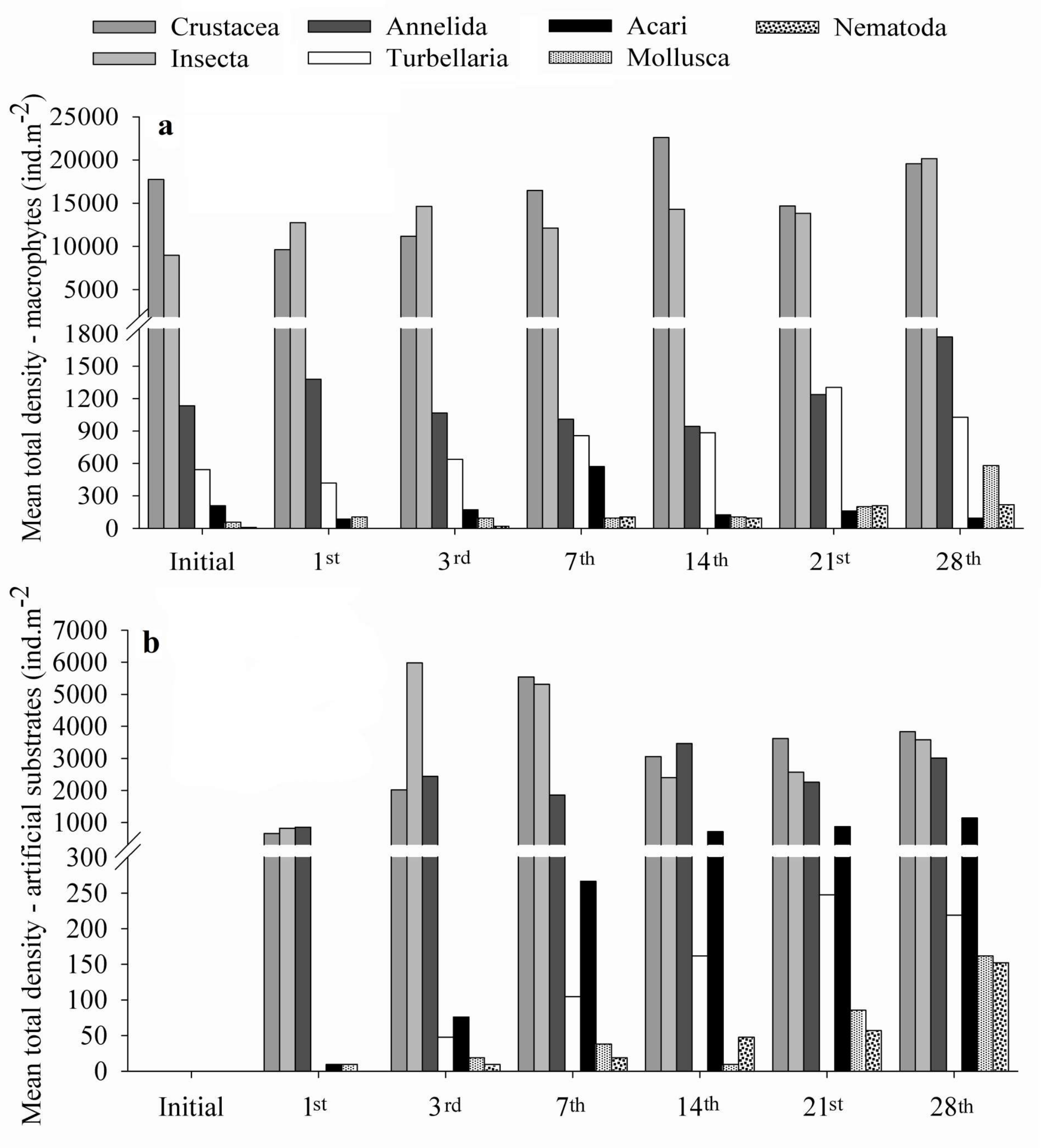

Figure 7. Mean (N = 3) of the total density (ind.m^{-2}) of macroinvertebrate taxa in macrophytes (a) and on the artificial substrate (b) on the plain lateral to the Guareí River, State of São Paulo, Brazil, on successive sampling days from December 2012 to January 2013. Note the difference in scales.

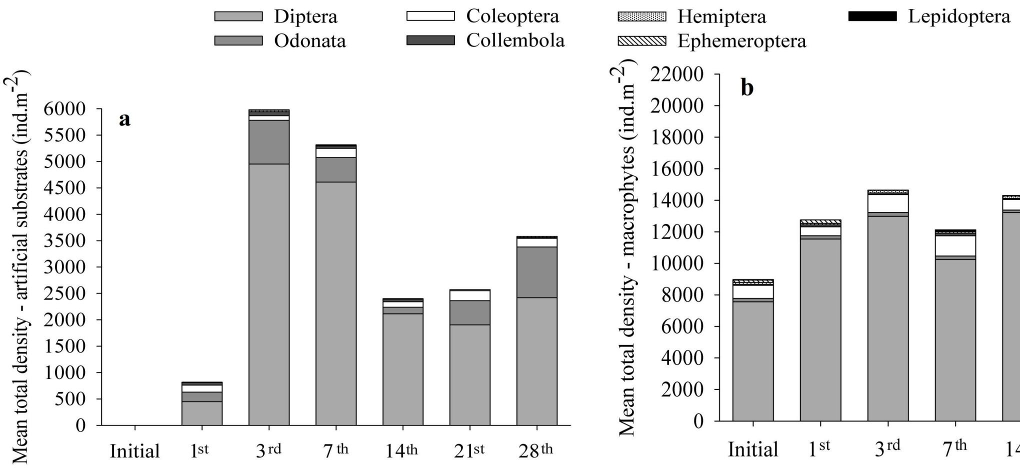

Figure 8. Mean (N = 3) of total density (ind.m^{-2}) of the different orders of Insecta associated with macrophytes (a) and with the artificial substrate (b) on the plain lateral to the Guareí River, State of São Paulo, Brazil, on successive sampling days from December 2012 to January 2013. Note the difference in scales.

According SIMPER analysis (similarity percentage analysis) the densities of Oligochaeta (contribution of 19.3%), Amphipoda (contribution of 15.4%) and Chironominae (contribution of 11.3%) were the main densities responsible for the similarity within the group shaped by the artificial substrate. In relation to the macroinvertebrates associated with macrophytes, Amphipoda (contribution of 22.3%), Chironominae (contribution of 12.7%) and Orthocladiinae (contribution of 11.0%) contributed significantly to the NMDS result (Figure 10a).

DISCUSSION

The artificial substrates used in this study were efficient for the colonization of macroinvertebrates, however their density did not follow an ascendant curve of increase of fauna from the incubation day until the end of the experiment. Structural and functional alterations in freshwater macroinvertebrate populations and biota communities are faster and usually occur as a function of short-term variability in environmental characteristics, such as rainfall, and the consequent change in water level and discharge, temperature and food availability (*e.g.*, input of allochthonous organic matter) (Resh *et al.* 2013). Thomazi *et al.* (2008) also reported that intense rainfall affects the colonization process because rains resulted in increased water movement and the consequent washing of invertebrates present in the environment, considerably reducing their number.

The taxa richness variation was low on the temporal scale. Conversely, the taxa density on the artificial substrate increased from the 1st to 7th day, showing a decline from the 7th to the 14th day, and a trend to a new increase on the 28th day. This is an indication that a new colonization process occurred and that ecological succession began on the 14th day. According to Connell & Slatyer (1977), succession in the majority of the communities is frequently interrupted by great perturbations occurring in nature (*e.g.*, fires, storms) and the process starts all over again. However, to study the occurrence of disturbances in nature is more complex than the theories show, because it can lead to a simplified idea of a predictable effect of perturbation on diversity (Fox 2013).

The biota response to disturbances can be better interpreted by examining the eventual resistance and resilience of the individuals. In previous studies that analyzed disturbance effects (*e.g.*, inundation and drought on freshwater macroinvertebrates), resistance is determined by measuring structural alterations in the community after the perturbation and resilience is defined as the capacity of recovery of the individuals and attaining the condition previous to the disturbance within a determined time scale (Melo *et al.* 2003, Fritz & Dodds 2004, Hershkovitz & Gasith 2013).

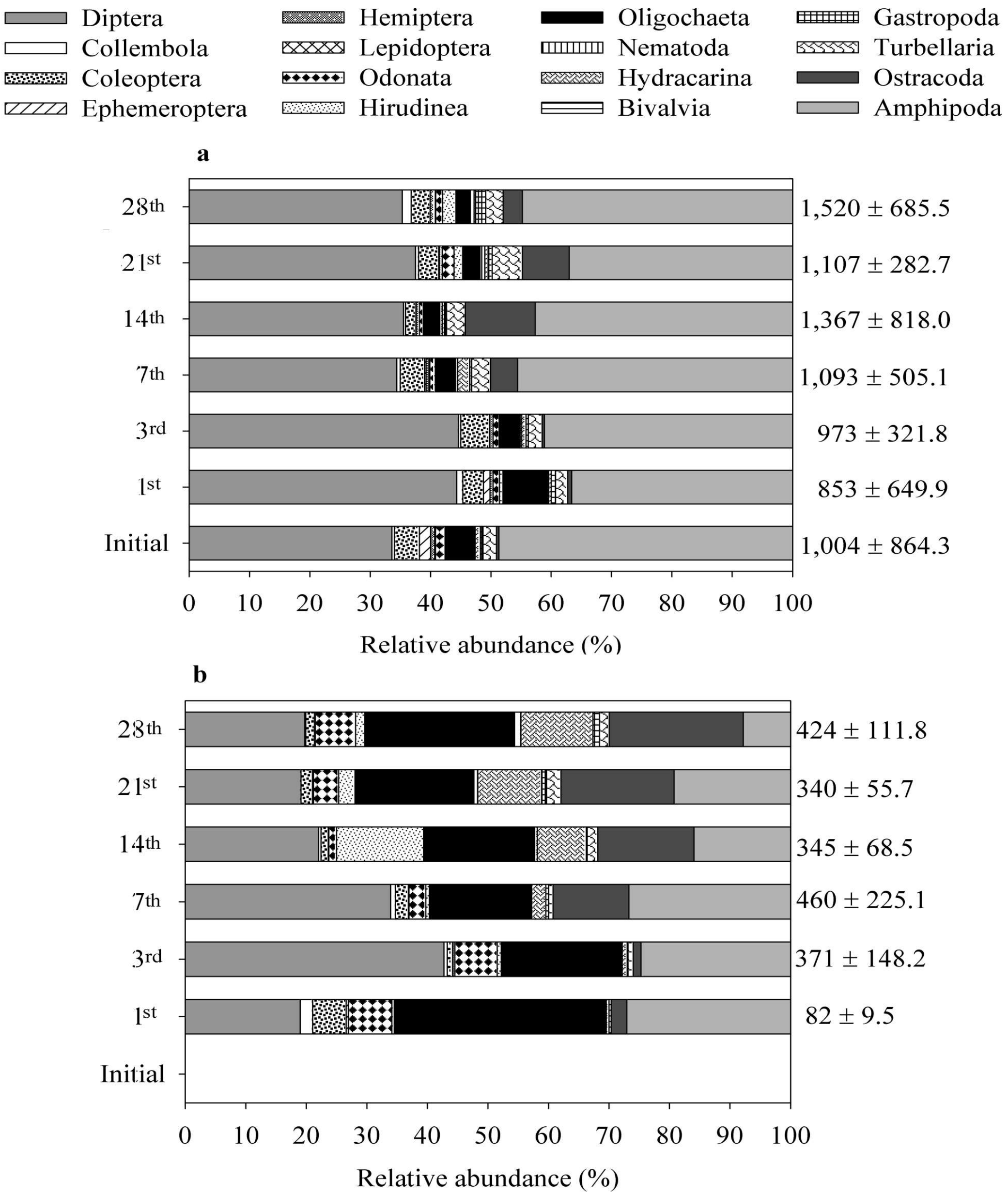

Figure 9. Mean (N = 3) of relative abundance (%), total abundance and standard-deviation (±) of macroinvertebrates associated with macrophytes (a) and on the artificial substrate (b) on the plain lateral to the Guareí River, State of São Paulo, Brazil, on successive sampling days from December 2012 to January 2013.

In our study, Chironomidae and Amphipoda can be considered resistant organisms, since both taxa were abundant on the artificial substrate and in the macrophytes on all sampling days. Conversely, in a study by Robinson (2012), these two taxa were less resistant to inundation, since they presented a significant reduction (> 70%) in their densities after the disturbance. Considering the eventual resilience, in the present study it was observed that the community recovered after ≅15 days in the macrophytes and after ≅ 21 days on the artificial substrate. Due to frequent precipitation during the study period, we cannot asseverate that these two respective periods are necessary for the recovery of equilibrium in macroinvertebrate density, since the duration may be different in a highly stable environment. Therefore, seasonal changes in macroinvertebrate abundance may affect the measurement of resilience capacity of individuals (Robinson 2012). Varying inundation magnitude can lead to different effects on community structure in an aquatic environment due to differences in life cycle, resistance and adaptation strategies of

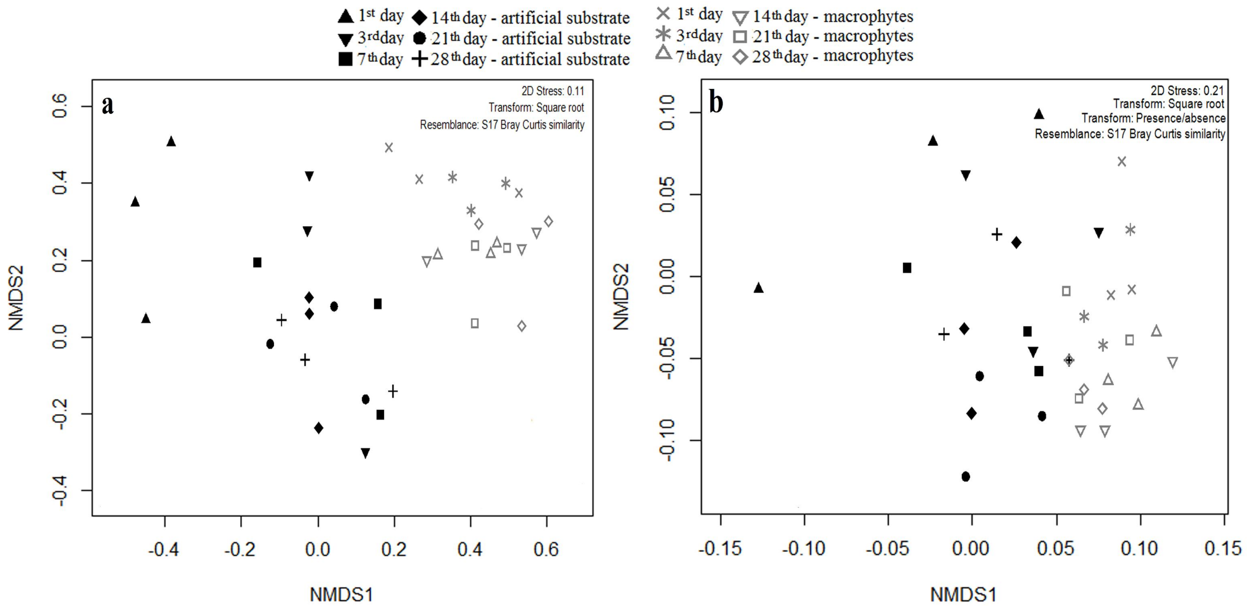

Figure 10. Non-metric multidimensional scaling (NMDS) analysis of density (ind.m^{-2}) (a) and composition (b) of macroinvertebrates associated with the artificial substrate and the macrophytes on the plain lateral to the Guareí River, State of São Paulo, Brazil, on successive sampling days from December 2012 to January 2013.

the organisms. Low and medium intensity inundation may reduce the density of aquatic communities. Conversely, high magnitude inundation may remove a determined population temporarily (Maltchick & Florin 2002). In the present study, inundation was probably of medium intensity, because only a diminution in the macroinvertebrate density was observed, without taxa exclusion. In relation to the fauna in the macrophytes, a fluctuation in density and taxa number values was seen in the temporal scale. The analysis of the maintenance of the macroinvertebrate community structure under disturbance can give an idea of how it is affected and re-established after perturbation (Connell & Slatyer 1977). To determine the intensity of the effects of hydrologic disturbance on aquatic biota requires a perception of the resistance threshold of each community or population to perturbations by inundation (Maltchick & Florin 2002).

According to the SIMPER analysis, the most abundant taxa on the artificial substrate were Oligochaeta, Amphipoda, and Chironominae, while Amphipoda, Chironominae and Orthocladiinae were higher in the macrophytes. These taxa were thus the most resistant to the effects of inundation on the plain lateral to the Guareí River. Also, Oligochaeta, Amphipoda and Chironominae, pioneer organisms important in the ecological succession, were observed on the artificial substrate from the beginning of the experiment. Pioneer taxa are less specialized organisms, change the habitat and allow colonization by other individuals which may extinguish the precursors in the ecological succession process (Carvalho & Uieda 2004). However, in the present study, Oligochaeta, Amphipoda and Chironomidae were observed on the artificial substrate on all the sampling days and no taxa change pattern was identified in the process. Thus, the macroinvertebrate ecological succession reported in the present study showed a pattern distinct from those reported in the literature.

Many studies have reported that Chironomidae and Oligochaeta are important colonizers of artificial substrates (Carvalho & Uieda 2004, Souza *et al*. 2008, Barker *et al*. 2014). However, on Amphipoda most information refers its taxonomy, biology, ecology, and their association with aquatic macrophytes and macro-algae (Jacobucci & Leite 2002, Saigo *et al*. 2009, Acosta & Prat 2011, Bastos-Pereira & Bueno 2013). A study on temporal and spatial patterns of macroinvertebrates associated with different floating macrophytes revealed that when the area covered by *R. natans* is large, the macroinvertebrate community is dominated by *Hyalella curvispina* amphipodes

(Fontanarrosa *et al*. 2013). Amphipoda individuals are commonly found in association with macrophytes and macro-algae (Jacobucci & Leite 2002) and present an herbivorous or herbivorous-detritivorous feeding habit (Jacobucci & Leite 2006, Saigo *et al.* 2009, Acosta & Prat 2011, Barker *et al.* 2014). This peculiarity of these organisms can explain the dominance of crustaceans in *R. natans* and *S. intermedia* stands*,* attaining approximately 50% of the relative abundance. Aquatic plants with low biomass and little developed roots and low capacity to retain particulate organic matter present a high number of associated herbivores and predators (Poi de Neiff & Neiff 2006).

Although amphipods were recorded on the artificial substrate, their relative abundance was lower than 35%. NMDS analysis showed a clear distinction between the density and composition of taxa associated with macrophytes compared to those on the artificial substrate. According to the SIMPER analysis, the percent contribution of Amphipoda to the NMDS result was higher in the macrophytes (22.3%) than on the artificial substrate (15.4%). The analysis pointed out taxa segregation on the artificial substrate and in the macrophytes on all the successive sampling days, confirming our working hypothesis concerning a difference in taxa composition on both substrates. No similarity trend in density values on either the natural and artificial substrates was observed at the end of the experiment, refuting our initial hypothesis.

A comparative study of macroinvertebrates associated with *Eichhornia crassipes* and an artificial substrate analog to its roots showed that the fauna structure differed in both substrates (Barker *et al.* 2014). Macrophyte roots provide a food source to some taxa, this being a determining factor in the selection of which plant to inhabit and which habitat is more useful to macroinvertebrates (Barker *et al.* 2014). Taxonomic composition (taxa presence or absence) in the present study showed that Culicidae, Tipulidae and Lepidoptera were exclusive in macrophytes, and Muscidae, Tabanidae, and Bivalvia were rare on the artificial substrate. Some lepidopterans and dipterans are shredder-herbivorous organisms (Newman 1991, Nachtrieb *et al.* 2011), which can explain their presence in plants. In this study, the artificial substrate supplied no food to herbivorous macroinvertebrates that feed on periphyton, because the supporting Styrofoam disks protected the polyester carpet samplers from light incidence and prevented alga colonization and growth. Thus, the macroinvertebrates associated with the artificial substrate had only adhered particulate organic matter and organisms from inferior trophic levels as a source of food and were a food source for top predators, such as Odonata larvae, the second most numerous aquatic insect after dipterans.

Although the feeding habits of macroinvertebrates supply important information for the understanding of their presence or absence at a determined site, there is yet much doubt concerning "true colonization" *vs*. "resources exploitation" in experiments using artificial substrates. Colonization can be characterized as complete ("true") only when taxa richness and density values on artificial substrates become stable or are similar to those of the surrounding community (Mackay 1992). However macroinvertebrates present a patch-like aggregate distribution in the environment that varies with colonization stage and taxonomic composition. Thus, artificial substrates distributed randomly in the environment can be ineffective in the precise estimation of macroinvertebrates in the ecosystem (Benoît*et al.* 1998).

According to Melo *et al.* (2003), inundation of aquatic environments cause a re-distribution of invertebrates and some sites loose individuals during the high water period, while others retain or receive organisms, acting as shelter. They also claim that habitats with high spatial heterogeneity furnish an ample range of shelters against hydraulic perturbations. Our sampling site was located within a preserved forest on the plain lateral to the Guareí River and presented great heterogeneity due to the presence of *R. natans* and *S. intermedia,* and also due to the riparian forest located on the temporary shores. Riparian vegetation contributes to increase the spatial heterogeneity of the aquatic environment, from the input of allochthonous matter to water bodies, and in the marginal zone of aquatic ecosystems it provides additional food resources and shelter to macroinvertebrates. Intrinsic characteristics of our sampling sites can have thus greatly diminished the effect of inundation pulses on the macroinvertebrate communities, considering that only a fluctuation in taxa density values was observed, not taxa exclusion.

The total density and taxonomic richness of macroinvertebrates associated with macrophytes was higher than that on the artificial substrate, refuting our initial hypothesis. In relation to taxonomic composition on both substrates our second hypothesis was confirmed, because the NMDS analysis pointed out taxa segregation on the artificial substrate and in the macrophytes on all the successive sampling days. Culicidae, Tipulidae and Lepidoptera were taxa exclusive in macrophytes, and Muscidae, Tabanidae, and Bivalvia were rare on the artificial substrate. Diptera and Amphipoda were abundant taxa in both substrates.

The hydrologic perturbation observed in the present study determined no fauna disappearance. Oscillations between an increase and a decline in density values in both substrates show that the macroinvertebrate fauna was maintained by a dynamic involving "de-colonization" and "re-colonization" (*i.e.*, emigration and immigration). Hydrologic disturbances in lotic ecosystems and marginal floodable areas must be considered not as a problem in the studies of macroinvertebrate colonization, but rather as an opportunity to investigate the influence and importance of stochastic and deterministic processes in the shaping of the structure of aquatic communities (Mackay 1992).

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REFERENCES

Acosta, R., & Prat, N. 2011. Trophic ecology of *Hyalella* sp. (Crustacea: Amphipoda) in a high Andes headwater river with travertine deposits. International Review of Hydrobiology, 96(3), 274-285. DOI: 10.1002/iroh.201111247

Albertoni, E. F., & Palma-Silva, C. 2006. Macroinvertebrados associados a macrófitas aquáticas flutuantes em canais urbanos de escoamento pluvial (Balneário Cassino, Rio Grande, RS). Neotropical Biology and Conservation, 1(2), 90-100.

Barker, J. E., Hutchens Jr., J. J., & Luken, J. O. 2014. Macroinvertebrates associated with water hyacinth roots and a root analog. Freshwater Science, 33(1), 159-167. DOI: 10.1086/674173

Bastos-Pereira, R., & Bueno, A. A. 2013. A new species of freshwater amphipod (Dogielinotidae, *Hyalella*) from southeastern Brazil. Nauplius, 21(1), 79-87.

Benoît, H. P., Post, J. R., Parkinson, E. A., Johnston, N. T. 1998. Colonization by lentic macroinvertebrates: evaluating colonization processes using artificial substrates and appraising applicability of the technique. Canadian Journal of Fisheries and Aquatic Sciences, 55(11), 2425-2435. DOI: 10.1139/f98-125

Booth, A. J., Kadye, W. T., & Wright, M. 2013. Rapid colonisation of artificial substrates by macroinvertebrates in a South African lentic environment. African Journal of Aquatic Science, 38(2), 175-183. DOI: 10.2989/16085914.2012.744687

Brederveld, R. J., Jähnig, S. C., Lorenz, A. W., Brunzel, S., Soons, M. B. 2011. Dispersal as a limiting factor in the colonization of restored mountain streams by plants and macroinvertebrates. Journal of Applied Ecology, 48(5), 1241-1250.

Cardinale, B. J., Palmer, M. A., Swan, C. M., Brooks, S., Poff, N. L. 2002. The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. Ecology, 83(2), 412-422.

Carvalho, E. M., & Uieda, V. S. 2004. Colonização por macroinvertebrados bentônicos em substrato artificial e natural em riacho de serra de Itatinga, São Paulo, Brasil. Revista Brasileira de Zoologia, 21(2), 287-293. DOI: 10.1590/S0101-81752004000200021

Carvalho, E. M., Uieda, V. S., & Motta, R. L. 2008. Colonization of rocky and leaf pack substrates by benthic macroinvertebrates in a stream in Southeast Brazil. Bioikos, 22(1), 37-44.

Clarke, K. R., & Gorley, R. N. 2006. PRIMER v6: User Manual/Tutorial. Plymouth: PRIMER-E: p. 192.

Clarke, K., & Warwick, R. M. 2001. Change in marine communities: an approach to statistical analysis and interpretation. 2nd ed. Plymouth: PRIMER-E.

Connell, J. H., & Slatyer, R. O. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. The American Naturalist, 111(982), 1119-1144.

Domínguez, E., & Fernández, H. R. 2009. Macroinvertebrados bentônicos sudamericanos. 1ª ed. Tucumán: Fund. Miguel Lillo: p. 654.

Fontanarrosa, M. S., Chaparro, G. N., & O'farrell, I. 2013. Temporal and spatial patterns of macroinvertebrates associated with small and medium-sized free-floating plants. Wetlands, 33, 47-63. DOI: 10.1007/s13157-012-0351-3

Fox, J. W. 2013. The intermediate disturbance hypothesis should be abandoned. Trends in Ecology & Evolution, 28(2), 86-92. DOI: 10.1016/j.tree.2012.08.014

Fritz, K. M., & Dodds, W. K. 2004. Resistance and resilience of macroinvertebrate assemblages to drying and flood in a tallgrass prairie stream system. Hydrobiologia, 527, 99-112.

Golterman, K. L., Clymo, R. S., & Ohmstad, M. A. M. 1978. Methods for physical and chemical analysis of freshwaters. 2nd ed. Oxford: Scientific Publications: p. 213.

Hershkovitz, Y., & Gasith, A. 2013. Resistance, resilience, and community dynamics in mediterranean-climate streams. Hydrobiologia, 719, 59-75. DOI: 10.1007/s10750-012-1387-3

Jacobucci, G. B., & Leite, F. P. P. 2002. Distribuição vertical e flutuação sazonal da macrofauna vágil associada a *Sargassum cymosum* C. Agardh, na praia do Lázaro, Ubatuba, São Paulo, Brasil. Revista Brasileira de Zoologia, 19(Supl.1), 87-100.

Jacobucci, G. B., & Leite, F. P. P. 2006. Biologia populacional das espécies de Ampithoidae (Crustacea, Amphipoda) associadas a *Sargassum filipendula* (Phaeophyta, Fucales) na praia da Fortaleza, Ubatuba, São Paulo, Brasil. Revista Brasileira de Zoologia, 23(4), 1207-1216.

Lodge, D. M. 1991. Herbivory on freshwater macrophytes. Aquatic Botany, 41, 195-224.

Mackay, R. J. 1992. Colonization by lotic macroinvertebrates: a review of processes and patterns. Canadian Journal of Fisheries and Aquatic Sciences, 49(3), 617-628. DOI: 10.1139/f92-071

Maltchik, L., & Florin, M. 2002. Perspectives of hydrological disturbance as the driving force of Brazilian semiarid stream ecosystems. Acta Limnologica Brasiliensia, 14(3), 35-41.

Melo, A. S., Niyogi, D. K., Matthaei, C. D., Townsend, C. R. 2003. Resistance, resilience, and patchiness of invertebrate assemblages in native tussock and pasture streams in New Zealand after a hydrological disturbance. Canadian Journal of Fisheries and Aquatic Sciences, 60(6), 731-739. DOI: 10.1139/f03-061

Mugnai, R., Nessimian, J. L., & Baptista, D. F. 2010. Manual de identificação de macroinvertebrados aquáticos do estado do Rio de Janeiro. 1ª ed. Rio de Janeiro, RJ: Technical Books: p. 174.

Nachtrieb, J. G., Grodowitz, M. J., & Smart, R. M. 2011. Impact of invertebrates on three aquatic macrophytes: American pondweed, Illinois pondweed, and Mexican water lily. Journal of Aquatic Plant Management, 49, 32-36.

Newman, R. M. 1991. Herbivory and detritivory on freshwater macrophytes by invertebrates: a review. Journal of the North American Benthological Society, 10(2), 89-114.

Odum, E. P., & Barrett, G. W. 2007. Fundamentos de Ecologia. 5ª ed. São Paulo: Cengage Learning: p. 612.

Poi de Neiff, A., & Neiff, J. J. 2006. Riqueza de especies y similaridad de los invertebrados que viven en plantas flotantes de la Planicie de Inundación del Río Paraná. Interciencia, 31(3), 220-225.

Pott, V. J., & Cervi, A. C. 1999. A família Lemnaceae Gray no Pantanal (Mato Grosso e Mato Grosso do Sul), Brasil. Revista Brasileira de Botânica, 22(2), 153-174. DOI:10.1590/S0100-84041999000200007

Pott, V. J., & Pott, A. 2000. Plantas aquáticas do Pantanal. Brasília: Embrapa: p. 404.

Resh, V. H., Bêche, L. A., Lawrence, J. E., Mazor, R. D., Mcelravy, E. P., O'dowd, A. P., Rudnick, D., Carlson, S. M. 2013. Long-term population and community patterns of benthic macroinvertebrates and fishes in Northern California Mediterranean-climate streams. Hydrobiologia, 719, 93-118. DOI: 10.1007/s10750-012-1373-9

Ribeiro, L. O., & Uieda, V. S. 2005. Estrutura da comunidade de macroinvertebrados bentônicos de um riacho de serra em Itatinga São Paulo, Brasil. Revista Brasileira de Zoologia, 22(3), 613-618.

Robinson, C. T. 2012. Long-term changes in community assembly, resistance, and resilience following experimental floods. Ecological Applications, 22(7), 1949-1961.

Saigo, M., Marchese, M., & Montalto, L. 2009. Hábitos alimentarios de *Hyalella curvispina* Shoemaker, 1942 (Amphipoda: Gammaridea) em ambientes leníticos de la llanura aluvial del río Paraná Medio. Natura Neotropicalis, 40(1,2), 43-59.

Sousa, W. P. 1984. The role of disturbance in natural communities. Annual Review of Ecology and Systematics, 15, 353-391.

Souza, A. H. F. F., Abílio, F. J. P., & Ribeiro, L. L. 2008. Colonização e sucessão ecológica do zoobentos em substratos artificiais no açude Jatobá I, Patos – PB, Brasil. Revista de Biologia e Ciências da Terra, 8(2), 125-144.

Thomazi, R. D., Kiifer, W. P., Ferreira Jr., P. D., & SÁ, F. S. 2008. A sucessão ecológica sazonal de macroinvertebrados bentônicos em diferentes tipos de atratores artificiais no rio Bubu, Cariacica, ES. Natureza on line, 6(1), 1-8.

Teixeira, C., & Kutner, M. B. 1962. Plankton studies in a mangrove environment. I - First assessment of standing stock and ecological factors. Boletim do Instituto Oceanográfico, 12, 101-124.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. Ecology, 75(1), 2-16.

Watson, A., & Barmuta, L. A. 2011. Feeding-preference trials confirm unexpected stable isotope analysis results: freshwater macroinvertebrates do consume macrophytes. Marine and Freshwater Research, 62(10), 1248-1257.

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