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Do changes in riparian zones affect periphyton growth and invertebrate colonization on rocky substrates in Atlantic Forest streams?

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ABSTRACT. We evaluated the growth of periphyton and colonization of sterilized cobbles by invertebrates in three coastal streams of the Atlantic Forest (Southeast Brazil) that differ in the conservation level of riparian zones. Because of differences in light availability and water temperature, we hypothesized the growth of periphytic algae would be higher in the most altered stream. Consequently, invertebrate assemblages would differ among streams. Cobbles with similar sizes were ashed and incubated for 7, 15, 30, 45 and 60 days in the studied streams. Despite periphyton growth was faster in the most altered stream, contents of chlorophyll-*a* did not differ among streams. A total of 954 individuals (98% insects) belonging to 36 taxa was found. Invertebrate density was higher and increased throughout the experiment in the preserved stream, while invertebrate biomass was higher on the initial sampling intervals (7 and 15 days). A stream effect on invertebrate assemblages was observed after the 15th day and 17 taxa were found only in the preserved stream. Leptophlebiidae (Ephemeroptera), Hydroptilidae, Helichopsychidae, Leptoceridae (Trichoptera) and Orthocladiinae (Diptera) showed specificities with the assemblages found in the preserved stream and no taxa proved to be an indicator of the assemblages found in the altered streams. These results showed that changes in the riparian zones of Atlantic Forest streams did not affect the content of chlorophyll-*a* on rocky substrates, but the growth of periphyton influenced the density and structural composition of invertebrate assemblages. Our findings partially support the proposed hypothesis and conform to the notion of the importance of periphyton community for the colonization of exposed substrates by invertebrates and for evaluating the consequences of anthropogenic changes in ecosystem functioning and aquatic communities.

KEYWORDS. Periphytic biomass, invertebrate assemblages, exposed substrates, anthropogenic changes, tropical streams.

RESUMO. As alterações nas zonas ripárias afetam o crescimento do perifíton e a colonização de substratos rochosos por invertebrados em riachos de Mata Atlântica? O crescimento do perifíton e a colonização de seixos esterilizados por invertebrados foram avaliados em três riachos costeiros de Mata Atlântica (Sudeste do Brasil) que diferem no estado de conservação das zonas ripárias. Devido a diferenças na disponibilidade de luz e temperatura da água, foi hipotetizado que o crescimento das algas perifíticas seria maior no riacho mais alterado. Consequentemente, as assembleias de invertebrados iriam diferir entre os riachos. Seixos com tamanhos similares foram incinerados e incubados por 7, 15, 30, 45 e 60 dias nos riachos estudados. Apesar do crescimento do perifíton ter sido mais rápido no riacho mais alterado, os teores de clorofila-*a* não diferiram entre os riachos. Um total de 954 indivíduos (98% insetos) pertencentes a 36 táxons foi encontrado. A densidade de invertebrados foi maior e aumentou ao longo do experimento no riacho preservado, enquanto a biomassa de invertebrados foi maior nos intervalos iniciais de amostragem (7 e 15 dias). Um efeito dos riachos nas assembleias de invertebrados foi observado após o 15º dia e 17 táxons foram encontrados somente no riacho preservado. Leptophlebiidae (Ephemeroptera), Hydroptilidae, Helichopsychidae, Leptoceridae (Trichoptera) e Orthocladiinae (Diptera) apresentaram especificidades com as assembleias encontradas no riacho preservado e nenhum táxon foi indicador das assembleias encontradas nos riachos alterados. Estes resultados demonstraram que alterações nas zonas ripárias de riachos de Mata Atlântica não afetaram os teores de clorofila-*a* em substratos rochosos, mas o crescimento do perifíton influenciou a densidade e a composição estrutural das assembleias de invertebrados. Estas observações suportam parcialmente a hipótese proposta e estão em conformidade com a noção da importância da comunidade perifítica para a colonização de substratos expostos por invertebrados e para a avaliação das consequências das alterações antrópicas no funcionamento dos ecossistemas e nas comunidades aquáticas.

PALAVRAS-CHAVE. Biomassa perifítica, assembleias de invertebrados, substratos expostos, alterações antrópicas, riachos tropicais.

Periphyton communities play an important role in the energy flow of freshwater ecosystems, with high ecological importance in the food chains of these environments (Feminella & Hawkins, 1995; Brito *et al*., 2006). These communities may influence the growth, development, survivorship and reproduction of many heterotrophic organisms, especially fish, aquatic insects and crustaceans (Moulton *et al*., 2004; Ceneviva-Bastos & Casatti, 2014).

Algae are the most-studied component of periphyton and are responsible for much of the gross primary production in stream ecosystems (Schneck *et al*., 2013; Moulton *et al*., 2015). The presence of these primary producers alters the structure of the aquatic environment; the algae remove nutrients from the water column, minimize the effects of water flow on exposed substrates, and contribute to sediment stabilization (Larned, 2010). The biomass of periphytic algae may be influenced by several abiotic factors including temperature, luminosity, substrate type, water flow and nutrient content (Fanta *et al*., 2010; Rosa *et al*., 2013). Some biotic factors may negatively affect periphyton biomass, such as herbivory and bioturbation caused by aquatic organisms (Wellnitz & Poff, 2012).

Allochthonous organic matter constitutes the main energy source for the metabolism of streams shaded by riparian vegetation (Vannote *et al*., 1980; Minshall *et al*., 1983). However, even with the predominance of leaf litter on the streambed, some studies have suggested that the autochthonous production of periphyton constitutes an important food resource for heterotrophic organisms in tropical streams (Brito *et al*., 2006; Li & Dudgeon, 2008; Lau *et al*., 2009). Because of the low quality of the allochthonous organic material available (high C:N ratio and toughness), periphytic algae are more palatable for consumers in these ecosystems (Burns & Walker, 2000).

The growth of periphyton facilitates the colonization of substrates by invertebrate scrapers, which have specialized mouthparts to feed on attached algae (Wallace & Webster, 1996). These organisms produce fine particulate organic matter through their feces, which can then be used as food resource by collectors (Cummins & Klug, 1979). Thus, the spatial distribution and biomass of periphyton can modify the structure of aquatic assemblages associated with different substrates, influencing the organisms that feed directly on these communities (Yafe *et al*., 2002; Quintans *et al*., 2009), shelter from predators and water flow, and feed on fine particulate organic matter deposited on the periphyton or produced by scrapers (Moulton *et al*., 2004; Wellnitz & Poff, 2012).

Changes in the riparian vegetation and nutrient input from agricultural activities or pollution sources may increase periphyton primary productivity and biomass in streams (Fanta *et al*., 2010). In reaches where the riparian vegetation was removed, periphyton algae can support higher production of aquatic invertebrate assemblages, in part because algae are assimilated more efficiently (Fuller *et al*., 2004). On the other hand, invertebrate diversity tends to be lower in altered streams (Oliveira & Callisto, 2010; Kleine *et al*., 2011; Mesa *et al*., 2013). According to Debenest *et al*. (2009), the input of herbicides in stream ecosystems reduces the biomass of periphytic algae, even in environments with good light and nutrient availability.

In this study, we evaluated the growth of periphyton and colonization of rocky substrates by invertebrate assemblages in three forest streams of the same watershed that differ in the conservation level of riparian zones. Assuming that changes in the riparian zones may alter light availability, water temperature and, consequently, the production of periphytic algae, we incubated sterilized cobbles on the substrate of the studied streams and determined the content of chlorophyll-*a* and the structural composition of invertebrate assemblages over 60 days. We hypothesized that the increase of periphytic biomass would be higher in the most altered stream and invertebrate assemblages associated with the exposed substrates would differ among streams.

MATERIAL AND METHODS

Study area. The studied streams are located in the headwaters of the Santa Maria da Vitória River, in the municipalities of Santa Leopoldina and Cariacica (Espírito Santo, SE Brazil). The study area contains several fragments of Atlantic Forest surrounded by agricultural areas and livestock pastures (Casotti *et al*., 2015). According with the Köppen's climate classification for Brazil, the regional climate is Humid Subtropical with Hot Summer (Cfa). Daily mean air temperatures and rainfall range between 19 – 28°C and 1000 – 1600 mm year^{-1} (Alvares *et al*., 2013). The forest canopy coverage in each stream was estimated by the analysis of photographs, taken with a digital camera (SONY Steady-Shot DSC-W610) positioned parallel and immediately above the water surface (Korhonen *et al*., 2006), using ImageJ Software (US National Institutes of Health, Bethesda, Maryland, USA).

Luxemburgo Stream (20°08'15.6"S – 40°35'44.4"W, 562 m a.s.l.) is located near small rural properties and extensive banana and *Eucalyptus* plantations. The reach selected for the study has low canopy cover (40%), and the substrate is composed basically of cobbles, sand, mud, and some leaf patches covered with fine sediment. Macuco Stream (20°01'23.1"S – 40°32'58.6"W, 593 m a.s.l.) is located in a well-preserved fragment of Atlantic Forest and has a developed canopy cover that shades almost 80% of the streambed. However, a monoculture of *Eucalyptus globulus* Labill. (~500 m^2) and a small rural property are located close to one of the margins of the stream. The substrate is quite heterogeneous and composed of cobbles, gravel, logs, and numerous leaf patches. Pau Amarelo Stream (20°15'41.3"S – 40°30'14.9"W, 426 m a.s.l.) is located in a protected area (Reserva Biológica Duas Bocas) and has a well-developed canopy cover that shades 90% of the streambed. The substrate is heterogeneous and composed mostly by cobbles and, in lower number, by gravel, logs and leaf patches. The studied streams formed a gradient of conservation level in the riparian

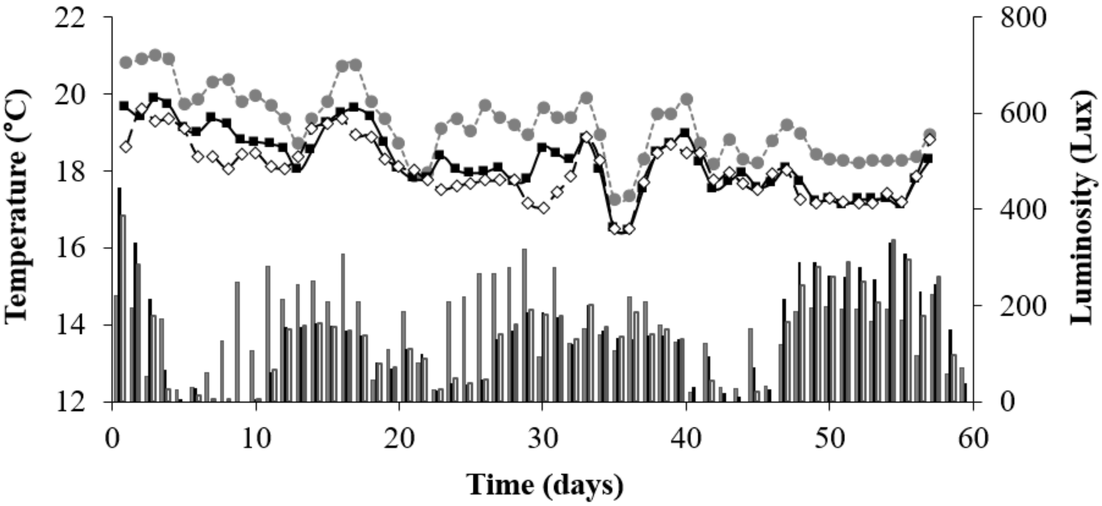

Fig. 1. Daily mean values of water temperature (lines) and luminosity (columns) in Luxemburgo (gray), Macuco (black) and Pau Amarelo (white) streams, state of Espírito Santo, Brazil during the experiment.

zones: Luxemburgo (altered) < Macuco (slightly altered) < Pau Amarelo (preserved).

During the experiment, which lasted from June to August 2012, the streams had well-oxygenated water, with slightly acid pH and low values of electrical conductivity (Tab. I). The initial content of Nitrogen and Phosphorus in the water ranged between 0.01 – 0.06 mg l^{-1} and 0.001 – 0.010 mg l^{-1}, respectively. Luminosity and water temperature were measured at 30-min intervals by temperature/light data loggers (HOBO UA-002-08, Onset Computer Corporation, Bourne, MA, USA) placed on the streambed of each stream. Daily mean water temperatures were higher in Luxemburgo than in Macuco and Pau Amarelo ($F_{2, 168} = 31.872$, $p < 0.001$; Fig. 1). The luminosity varied between 157 ± 10 lux in Luxemburgo stream and 128 ± 13 lux in Pau Amarelo stream, but did not differ significantly among streams ($F_{2, 168} = 1.26$, $p = 0.286$).

Experimental design. On June 6th, 2012, four quadrats of 1 m^2 were fixed on the streambed of each stream, using iron bars and colored markers. In each quadrat, we incubated five cobbles of similar shape and size that had been previously collected from the streams, washed and ashed (550°C, 2 h) in the laboratory. These cobbles were used as substrate for the growth of periphytic algae and colonization of aquatic invertebrates. At intervals of 7, 15, 30, 45 and 60 days of incubation, periphyton and invertebrate assemblages were sampled from one incubated cobble in each quadrat, i.e., four cobbles (replicates) in each stream.

At each sampling interval, the invertebrates associated with each incubated cobble were sampled with a Surber sampler (0.09 m^2, 250 µm mesh). These samples were placed in individual plastic bags and taken to the laboratory. Then, each cobble was removed from the respective quadrat for periphyton sampling. Cobbles were placed on individual plastic trays containing stream water and brushed carefully. The extract retained in the trays was stored in covered centrifuge tubes (50 ml) under refrigeration and taken to the laboratory. After the periphyton was removed, the active surface area of each cobble, i.e., the area excluding the lower surface, was determined following Rosa *et al*. (2013) (Luxemburgo: 174.3 ± 7.7 cm^2; Macuco: 166.26 ± 10.1 cm^2; Pau Amarelo: 154.20 ± 7.5 cm^2; $F_{2, 57} = 1.39$, $p = 0.255$).

Sample processing. The chlorophyll-*a* content was used as an indicator of the growth of periphytic algae on each incubated cobble. In the laboratory, the volume of each periphyton sample, i.e., the extract removed from each cobble (four replicates per sampling interval in each stream), was determined, and then the sample was filtered through

Tab. I. Water physical and chemical properties (mean, maximum and minimum values) of Luxemburgo, Macuco and Pau Amarelo streams, state of Espírito Santo, Brazil during the experiment (n = 6 *in situ* measurements; *, one single measurement).

	Luxemburgo		Macuco		Pau Amarelo	
	Mean	Range	Mean	Range	Mean	Range
Width (m)	6.76	6.40 – 7.30	3.42	2.40 – 4.10	5.78	5.00 – 6.70
Depth (m)	0.27	0.23 – 0.34	0.14	0.09 – 0.20	0.23	0.21 – 0.26
Discharge (m^3 s^{-1})	0.24	0.10 – 0.26	0.10	0.07 – 0.14	0.43	0.31 – 0.56
Conductivity (µS cm^{-2})	26.12	24.60 – 28.10	21.55	19.40 – 26.39	29.86	27.60 – 35.80
Dissolved O_2 (mg l^{-1})	8.74	8.59 – 8.89	8.75	8.70 – 8.79	8.39	7.89 – 8.64
pH	6.34	6.14 – 7.16	6.59	6.17 – 7.25	6.64	6.20 – 6.80
Total N (mg l^{-1})*	0.06		0.04		0.01	
Total P (mg l^{-1})*	0.010		0.004		<0.001	

fiberglass filters (GF/F Glass Microfiber Filter, 47 mm, Whatman, Shrewsbury, MA, USA; 0.7 µm pore size). The filters were stored individually in covered Petri dishes and frozen (-20°C). Posteriorly, the pigments were extracted in 90% acetone for 24 h at 4°C and the filters slurry was centrifuged at 1000 g for 5 min. Analysis of chlorophyll-*a* was performed using a multi-wavelength spectrophotometer following EPA's Method 446.0 (Arar, 1997).

Invertebrate samples were washed over 250-µm sieves and fixed with 70% ethanol. The samples were then sorted, and the invertebrates identified with a stereoscopic microscope (32x) to the lowest possible taxonomic level (Pes *et al.*, 2005; Mugnai *et al.*, 2010; Trivinho-Strixino, 2011). Invertebrate taxa were assigned to functional feeding groups, following specific classifications for Brazil (Cummins *et al.*, 2005; Baptista *et al.*, 2006) and oven-dried (60°C, 72 h) for biomass determination (0.01 mg).

Data analysis. Data for the density (ind cm^{-2}) and biomass (mg cm^{-2}) of the invertebrate assemblages and content of chlorophyll-*a* (µg cm^{-2}) were compared by mixed-design analysis of variance models (split-plot ANOVA), using the streams as a fixed factor and sampling intervals as a random factor. When the assumption of sphericity has been violated (Mauchly test: $p < 0.05$), degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon < 0.75$). Post-hoc comparisons were carried out using Bonferroni test (Zar, 2010). Invertebrate richness was compared among streams by Mao-Tau sample-based rarefaction curves with 95% confidential intervals (Colwell *et al.*, 2004). To assess the spatial structures of invertebrate assemblages sampled in the studied streams at different sampling intervals, a canonical analysis of principal coordinates (CAP; Anderson & Willis, 2003) was performed using the Bray-Curtis resemblance distance matrix (log-transformed data, 999 permutations). Invertebrate taxa with a correlation greater than 0.3 were overlaid as vectors on the CAP plot. A PERMANOVA was used to test for significant differences among the assemblages found in each stream. The Analysis of Indicator Species (Dufrêne & Legendre, 1997) was used to determine if any taxon was characteristic of one group of samples (stream). All statistical analyses were performed using R (R Development Core Team, Vienna, Austria) and Primer 6.0 (Primer-E Ltd, Plymouth, UK).

RESULTS

In Luxemburgo, the contents of chlorophyll-*a* indicated rapid growth of periphytic algae on the first 15 days of the experiment (Fig. 2). These values showed low variation until the 45th day and decreased during the last sampling interval. In Macuco and Pau Amarelo, periphyton growth was more continuous throughout the experiment and the highest values were observed during the last sampling interval. However, these values increased faster in Macuco during the first 15 days, while high values were observed in Pau Amarelo after the 45th and 60th days (Fig. 2). The content of chlorophyll-*a* did not differ among streams, but, except for Luxemburgo on day 60, values observed after the first week of the experiment were significantly lower than those of other sampling intervals (Tab. II). The interaction of these two factors (streams × sampling intervals) was also significant.

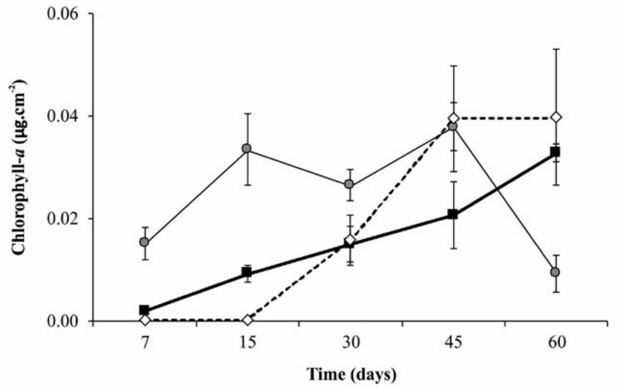

Fig. 2. Contents of chlorophyll-*a* (mean ± SE) on the cobbles incubated in Luxemburgo (thin solid line, gray circles), Macuco (thick solid line, black squares) and Pau Amarelo (dashed line, white diamonds) streams, state of Espírito Santo, Brazil.

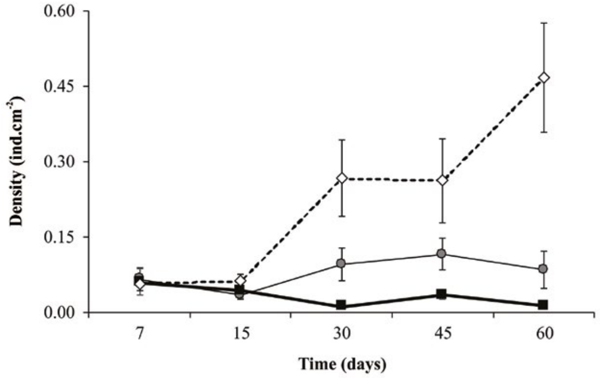

Fig. 3. Values (mean ± SE) of invertebrate density associated with cobbles in Luxemburgo (thin solid line, gray circles), Macuco (thick solid line, black squares) and Pau Amarelo (dashed line, white diamonds) streams, state of Espírito Santo, Brazil.

The density of invertebrates found associated with cobbles in the studied streams was similar on the first 15 days of the experiment. In Pau Amarelo, values of invertebrate density increased between the 15th and the 60th day (0.06 ± 0.01 up to 0.47 ± 0.10 ind cm^{-2}; Fig. 3). These values were intermediate in Luxemburgo (0.04 ± 0.01 up to 0.12 ± 0.03 ind cm^{-2}) and low in Macuco, in which the maximum values were observed after the first 7 days of the experiment (0.06 ± 0.02 ind cm^{-2}). The values of invertebrate density observed in Pau Amarelo were significantly higher than those observed in Luxemburgo and Macuco; values on the initial sampling intervals (7 and 15 days) were lower than on more advanced colonization stages (30, 45 and 60 days; Tab. II). Differences were also observed on the interaction of both factors. In Luxemburgo and Macuco, the maximum values of invertebrate biomass were observed after the first 7 days of incubation (0.04 ± 0.01 and 0.03 ± 0.01 mg cm^{-2}, respectively; Fig. 4), while in Pau Amarelo maximum values were observed on the 15th day (0.09 ± 0.01 mg cm^{-2}).

Tab. II. Results from mixed-design analysis of variance models (split-plot ANOVAs) comparing the content of chlorophyll-*a*, and the values of density and biomass of invertebrates associated with cobbles in Luxemburgo, Macuco and Pau Amarelo streams, state of Espírito Santo, Brazil. Streams were used as a fixed factor and sampling intervals as a random factor. Degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon < 0.75$).

	df	MS	F	p
Chlorophyll- *a* content				
Between-subjects				
Stream	2	0.000	1.51	0.27
Error	9	0.000		
Within-subjects				
Sampling interval	1	0.004	16.09	< 0.01
Stream × Samp. interval	2	0.002	6.64	0.02
Error	9	0.000		
Invertebrate density				
Between-subjects				
Stream	2	0.196	35.56	< 0.01
Error	9	0.006		
Within-subjects				
Sampling interval	1	0.143	14.75	< 0.01
Stream × Samp. interval	2	0.141	14.62	< 0.01
Error	9	0.010		
Invertebrate biomass				
Between-subjects				
Stream	2	0.005	9.81	< 0.01
Error	9	0.000		
Within-subjects				
Sampling interval	1	0.005	8.54	0.01
Stream × Samp. interval	2	0.000	0.20	0.82
Error	9	0.001		

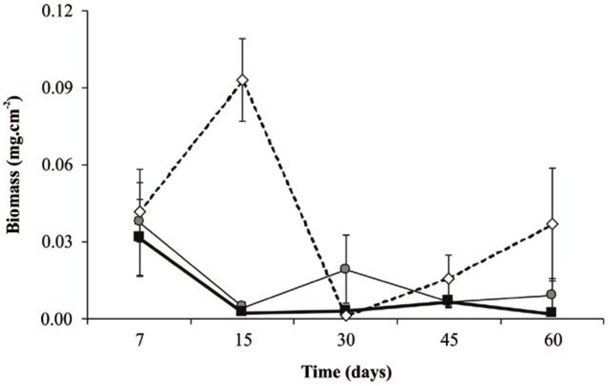

Fig. 4. Values (mean ± SE) of invertebrate biomass associated with cobbles in Luxemburgo (thin solid line, gray circles), Macuco (thick solid line, black squares) and Pau Amarelo (dashed line, white diamonds) streams, state of Espírito Santo, Brazil.

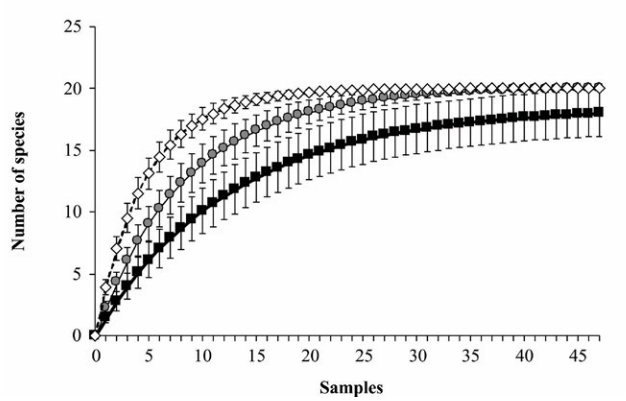

Fig. 5. Species accumulation curves (Mao-Tau sampled based rarefaction with 95% confidence intervals) of invertebrates associated with cobbles in Luxemburgo (thin solid line, gray circles), Macuco (thick solid line, black squares) and Pau Amarelo (dashed line, white diamonds) streams, state of Espírito Santo, Brazil.

Invertebrate biomass was higher in Pau Amarelo than in Luxemburgo and Macuco; values observed on the 15th day of the experiment were higher than those observed on other sampling intervals (Fig. 4; Tab. II). The interaction of both factors was not significant.

The richness of invertebrate assemblages varied among streams and the number of taxa was higher in Pau Amarelo (29), intermediate in Macuco (26) and lower in Luxemburgo (24). Taxonomic richness estimates using sample rarefaction curves showed that Mao-Tau estimator stabilized after approximately 20 samples in Pau Amarelo and 35 samples in Luxemburgo (Fig. 5). In Macuco, the estimator did not stabilize but, after 40 samples, the estimated numbers of taxa were similar to other streams.

In total, 954 invertebrates belonging to 36 taxa were found (Tab. III); the Class Insecta accounted for 98%. The number of organisms found associated with cobbles was higher in Pau Amarelo (579 ind.), intermediate in Luxemburgo (277 ind.) and lower in Macuco (98 ind.). Chironominae (Diptera) was the most abundant taxon in Luxemburgo and Pau Amarelo (37 and 42% respectively), while Simuliidae (Diptera) dominated invertebrate assemblages in Macuco

Tab. III. Density (ind cm^{-2}) of the invertebrate taxa associated with cobbles in Luxemburgo, Macuco and Pau Amarelo streams, state of Espírito Santo, Brazil (FFG, functional feeding group; Scr., scrapers; Gat.-Col., gathering-collectors; Pre., predators; Shr., shredders; Fil.-Col., filtering-collectors; -, absent; *, multiple feeding habits; ?, no information available).

Taxa	FFG	Streams		
		Luxemburgo	Macuco	Pau Amarelo
COLLEMBOLA	Gat.-Col.	0.008	0.006	0.008
EPHEMEROPTERA				
Baetidae	Scr.	0.216	0.070	0.337
Leptohyphidae	Scr.	0.203	0.066	0.334
Leptophlebiidae	Gat.-Col.	0.023		0.245
ODONATA				
Calopteterygidae	Pre.	0.006		
Gomphidae	Pre.	0.006	0.013	
Libellulidae	Pre.	0.005	0.008	
Megapodagrionidae	Pre.	0.044	0.053	0.075
PLECOPTERA				
Perlidae	Pre.	0.005	0.034	0.042
HEMIPTERA				
Gerridae	Pre.		0.006	0.004
Naucoridae	Pre.	0.015		
Veliidae	Pre.	0.014	0.021	0.052
COLEOPTERA				
Elmidae (larva)	Gat.-Col.	0.167	0.036	0.230
Elmidae (adult)	Scr.	0.021	0.012	
Psephenidae	Scr.			0.006
TRICHOPTERA				
Calamoceratidae				
Phylloicus major	Shr.	0.006	0.006	0.019
Glossosomatidae	Scr.			0.020
Helichopsychidae	Scr.	0.006		0.206
Hydropsychidae	Fil.-Col.	0.031	0.014	0.133
Hydroptilidae	Scr.			0.104
Leptoceridae	Gat.-Col./Shr.			0.083
Odontoceridae	Scr.		0.009	0.012
Philopotamidae	Fil.-Col.	0.026	0.006	0.007
Polycentropodidae	Fil.-Col.	0.020	0.009	0.040
DIPTERA				
Ceratopogonidae	Pre./Gat.-Col.		0.007	0.021
Chironomidae				
Chironominae	*	0.594	0.085	1.863
Stenochironomus	Shr.		0.007	
Orthocladiinae	*	0.048	0.018	0.281
Tanypodinae	Pre.	0.026	0.020	0.084
Empididae	Pre.		0.014	0.045
Psychodidae	Gat.-Col.			0.017
Simuliidae	Fil.-Col.	0.064	0.122	0.066
Tipulidae	Pre./Shr.		0.005	
LEPIDOPTERA				
Pyralidae	?			0.006
ANNELIDA				
Oligochaeta	Gat.-Col.	0.018	0.007	0.042
ACARINA				
Hydracarina	Pre.	0.020	0.006	0.033
Total abundance		277	98	579

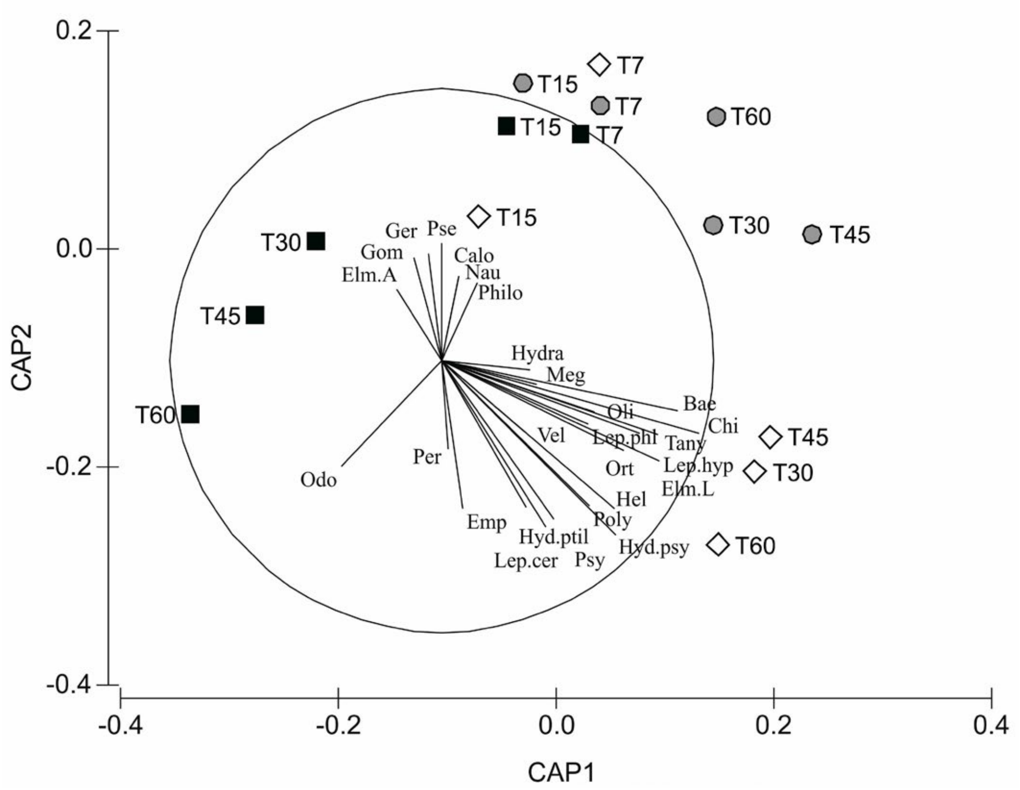

Fig. 6. Canonical analysis of principal coordinates (CAP) of invertebrates associated with cobbles in Luxemburgo (gray circles), Macuco (black squares) and Pau Amarelo (white diamonds) streams, state of Espírito Santo, Brazil. Only taxa vectors with correlations >0.3 are included in the plot. T7, T15, T30, T45 and T60: sampling intervals (Bae: Baetidae; Calo, Calopterygidae; Chi, Chironominae; Elm.A, Elmidae adult; Elm.L, Elamidae larva; Emp, Empididae; Ger, Gerridae; Gom, Gomphidae; Hel, Helichopsychidae; Hydra, Hydracarina; Hyd.psy, Hydropsychidae; Hyd.ptil, Hydroptilidae; Lep.cer, Leptoceridae; Lep.hyp, Leptohyphidae; Lep.phl, Leptophlebiidae; Meg, Megapodagrionidae; Nau, Naucoridae; Odo, Odontoceridae; Oli, Oligochaeta; Ort, Orthocladiinae; Per, Perlidae; Philo, Philopotamidae; Poly, Polycentropodidae; Pse, Psephenidae; Psy, Psychodidae; Tany, Tanypodinae; Vel, Veliidae).

(18%). Leptohyphidae and Baetidae (Ephemeroptera) exhibited high densities in all streams (7 – 13%), and Elmidae larvae (Coleoptera) exhibited higher densities in Luxemburgo (23%). The maximum values of relative biomass of scrapers were observed after 15 and 30 days in Pau Amarelo and Macuco (42 and 38%, respectively), while in Luxemburgo maximum values were observed after 60 days (60%). This was the only sampling interval in which scrapers accounted for more than 50% of the biomass of the invertebrate assemblages associated with cobbles.

The CAP ordination plot revealed that invertebrate assemblages on the initial sampling intervals formed a separate group (Fig. 6), while the assemblages sampled after the 15th day in each stream formed individual clusters. The stream effect on invertebrate assemblages was also supported by the PERMANOVA results (Pseudo-F = 2.21, $p < 0.01$). Comparing to the initial assemblages of studied streams that were structured by few invertebrate taxa (Philopotamidae [Trichoptera], Naucoridae [Hemiptera], Calopterygidae [Odonata] and Psephenidae [Coleoptera]), the assemblages sampled in Pau Amarelo after the 15th day were structured by a high number of taxa (17), which showed high densities or were found only in this stream (Fig. 6). However, the Analysis of Indicator Species indicated that only five taxa showed specificities with the assemblages found in Pau Amarelo (Tab. IV). No taxa proved to be an indicator of the assemblages found in Luxemburgo and Macuco.

Tab. IV. Results of the analysis of indicator species for the assemblages associated with cobbles in Pau Amarelo stream, state of Espírito Santo, Brazil. Only taxa with significant results are shown.

Taxa	Indicator Value	p - value
Leptophlebiidae	0.770	0.005
Hydroptilidae	0.742	0.005
Helichopsychidae	0.661	0.005
Leptoceridae	0.592	0.005
Orthocladiinae	0.667	0.005

DISCUSSION

Invertebrate assemblages found associated with the exposed substrates differed among the studied streams. After the initial stage of the experiment (first 15 days), cobbles incubated in Pau Amarelo were colonized by a high number of invertebrate taxa and the values of density increased until the end of the experiment. In Luxemburgo, invertebrate assemblages were dominated by taxa that are tolerant (Baetidae and Elmidae) or resistant (Chironominae) to anthropogenic changes, while lowest levels of invertebrate densities and taxa richness were observed in Macuco.

In spite of the conservation level in the riparian zones did not influence the biomass of periphyton, measured indirectly through the content of chlorophyll-*a*, the growth of periphytic algae was faster in the most altered stream. Shading is one of the main factors controlling periphyton biomass (Rier *et al*., 2014), and sunlight may enhance the effects of leaf-litter leachates on algae growth (Lovatt *et al*., 2014). In spite of the differences in canopy cover, luminosity levels did not differ between the streams. However, during most of the experiment, mean daily luminosity was higher in Luxemburgo. The warm water temperatures in this stream are probably related to changes in the riparian vegetation that allowed more sunlight to reach the stream channel. Bowler *et al*. (2012), working in European streams, observed that water temperatures were positively correlated with light input.

The rapid growth of periphyton in Luxemburgo probably resulted from higher water temperature, luminosity and dissolved nutrients in the water column. Temperature is a key factor in the development of algal biomass because it regulates cellular metabolism, tending to optimize growth rates (Bowes *et al*., 2007). Normally, nitrogen and phosphorus act as limiting nutrients for algae and periphyton growth in stream ecosystems (Murdock *et al*., 2011; Rosa *et al*., 2013). In streams located near agricultural areas, the periphyton production can be increased by input of fertilizers leached from surrounding areas (Conley *et al*., 2009). On the other hand, as we did not observe any change in water flow and substrate revolvement during the experiment, the decrease in the content of chlorophyll-*a* observed on the end of the experiment in Luxemburgo may be related to an increase in consumption of algae by invertebrate scrapers, which after 60 days represented more than 50% of the biomass of invertebrates found in Luxemburgo. According to Kiffney *et al*. (2003), Moulton *et al*. (2004) and Murdock *et al*. (2011), herbivory by aquatic insects, crustaceans and fish can significantly reduce algal biomass in streams by a top-down control of periphyton communities (Winkelmann *et al*., 2014).

In Pau Amarelo, the development of periphytic algae directly influenced the invertebrate density on the incubated cobbles, since periphyton communities provide a more stable habitat and food resources (Benetti *et al*., 2014). The elevated densities of chironomid larvae in the studied streams reflect the ability of rapid substrate colonization of this group, which includes species with different feeding habits that occur in ecosystems with a wide range of environmental conditions (Rosa *et al*., 2011). According to Oliveira & Nessimian (2010), chironomid larvae may feed on fine particulate organic matter, algae and small invertebrates associated with periphyton. The high abundances of larvae of Elmidae in Luxemburgo and Pau Amarelo could be explained by their morphological adaptations that allow them to attach to and feed on substrates under high-flow conditions (Elliott, 2008).

Contrary to our expectations, the biomass of aquatic invertebrates did not increase over the experiment. In addition, the values varied widely in Pau Amarelo. These results suggest that other factors, not directly related to periphyton growth, and also influenced the colonization of cobbles by invertebrates. Predation, for example, might have been an important structuring factor for the assemblages (McIntosh & Townsend, 1996; Fairchild & Holomuzki, 2005; Holomuzki *et al*., 2010). The studied streams contain macroconsumers (crabs and shrimps) that may control invertebrate communities, as observed by Lourenço-Amorim *et al*. (2014) and Costa *et al*. (2016) in Atlantic Forest streams located in the state of Rio de Janeiro and Espírito Santo, Brazil.

Comparing with the initial sampling intervals, the structural composition of invertebrate assemblages after the first month of the experiment differed more in Pau Amarelo. Since the growth of periphyton increased substantially after the 15th day in this stream, the exposed substrate changed continuously during sampling intervals (Munn *et al*., 2010), meeting the requirements of more different taxa. Pau Amarelo stream has a heterogeneous streambed inhabited by abundant and diverse assemblages of invertebrates (Flávio Mendes, pers. comm.), which may have acted as sources of colonizing organisms for the incubated cobbles (Bonada *et al*., 2006). Moreover, four taxa that were proved to be indicators of the invertebrate assemblages in this stream belong to Ephemeroptera and Trichoptera, which are normally abundant in natural streams, while they are found in reduced abundances or absent in streams with different levels of alterations (Amaral *et al*., 2015).

In conclusion, our results showed that changes in the riparian zones of Atlantic Forest streams did not affect the content of chlorophyll-*a* on rocky substrates but periphytic algae grew faster in the most altered stream. The growth of periphyton influenced the density and structural composition of invertebrate assemblages associated with cobbles, because differences were observed only after the initial sampling intervals, when values of chlorophyll-*a* increased substantially. These results partially support the proposed hypothesis and conform to the notion of the importance of periphyton communities for the colonization of exposed substrates by aquatic invertebrates. In addition to herbivory, other species interactions, such as predation, may also have influenced the colonization process. The relationship between the production of periphytic algae and invertebrate assemblages is important for understanding energy flow in forest streams, and at the same time, for evaluating the consequences of anthropogenic changes in ecosystem functioning and aquatic communities.

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