Chironomidae (Diptera) larvae associated to *Eucalyptus globulus* and *Eugenia uniflora* leaf litter in a subtropical stream (Rio Grande do Sul, Brazil)

Larvas de Chironomidae associadas a folhas em decomposição de *Eucalyptus globulus* e *Eugenia uniflora* em um riacho Subtropical (Rio Grande do Sul, Brasil)

Hepp, LU.[1], Biasi, C.[1], Milesi, SV.[1], Veiga, FO.[1] and Restello, RM.[1,2]

[1]Laboratório de Biomonitoramento, Departamento de Ciências Biológicas,
Universidade Regional Integrada do Alto Uruguai e das Missões, Campus de Erechim,
Av. Sete de Setembro, 1621, CEP 99700-000, Erechim, RS, Brazil
e-mail: lhepp@uri.com.br; crisbiasi@yahoo.com.br; silvia_milesi@yahoo.com.br; nando3b@hotmail.com

[2] Programa de Pós-Graduação em Ecologia, Universidade Regional Integrada do Alto Uruguai e das Missões,
Campus de Erechim, Av. Sete de Setembro, 1621, CEP 99700-000, Erechim, RS, Brazil
e-mail: rrozane@uri.com.br

Abstract: In low order streams, the input of allochthonous organic matter is the main resource of energy and shelter for the aquatic organisms. The aim of this work was to evaluate the structure and composition of Chironomidae fauna associated with decomposing leaves of *Eucalyptus globulus* and *Eugenia uniflora* vegetal breakdown, and to investigate the effect of leaves polyphenols contents in the leaf litter colonization by the organisms. In a second-order stream, leaf litter of *E. globulus* and *E. uniflora* were enclosed in 48 litter bags (24 of each species) with 3 ± 0.1 g of dry leaves. After 3, 6, 14, 21, 28, 35, 42 and 49 days, the litter bags were removed for the chironomids identification and analysis of the leaf litter. The leaf breakdown rates were different between both species. Fifteen genera of Chironomidae were found; *Rheotanytarsus* and *Thienemanniela* sp3 were the most frequent and abundant genera. No significant difference between Chironomidae communities of both vegetal species was detected; however the composition of FFG (Functional Feeding Groups) differed during the study period. A negative relation between polyphenols contents and larvae abundance was observed. The knowledge about the chemicals characteristics of the vegetal species can be an important factor in the comprehension of exotics plants effects on the aquatic communities.

Keywords: aquatic insects, decomposition, native and exotic species.

Resumo: Em riachos de pequena ordem, o material vegetal alóctone é a principal fonte de energia e abrigo para os organismos aquáticos. Este trabalho teve por objetivo avaliar a estrutura e composição da fauna de Chironomidae associada ao detrito vegetal de *Eucalyptus globulus* e *Eugenia uniflora*, além de avaliar os efeitos dos teores de polifenóis presentes nas folhas das espécies sobre a colonização dos organismos. Foram incubados em um riacho de segunda ordem 48 pacotes de folhas, sendo 24 para cada espécie, contendo 3 ± 0,1 g de folhas secas. Após 3, 6, 14, 21, 28, 35, 42 e 49 dias foram retirados pacotes de folhas de ambas as espécies para identificação dos organismos e análise do detrito. A taxa de decomposição foi diferente entre ambas as espécies estudadas. Foram identificados 15 gêneros de Chironomidae, sendo *Rheotanytarsus* e *Thienemanniela* sp3 os mais freqüentes e abundantes. Não houve diferença significativa entre a comunidade de Chironomidae em ambas as espécies vegetais, porém a composição dos grupos de alimentação funcional diferiu durante o período de estudo. Foi observada uma relação negativa entre os teores de polifenóis e a abundância das larvas. O conhecimento das características químicas das espécies vegetais pode ser um fator importante para auxiliar na compreensão dos efeitos de plantas exóticas sobre as comunidades aquáticas.

Palavras-chave: insetos aquáticos, decomposição, espécie nativa e exótica.

Biological Limnology

1. Introduction

Low-order streams depend on the riparian vegetation as energy source. In these environments, the input of allochthonous organic matter (leaves, wood, fruits, among others) is responsible for the energy supply and maintenance of the trophic chain (Vannote et al., 1980; Gonçalves-Jr et al., 2006a). Furthermore, the leaf packs in aquatic environments act as shelter for the majority of macroinvertebrates (Wantzen and Wagner, 2006).

The leaf decomposition in the streams is a continuous process that involves bacteria, fungi and macroinvertebrates (Graça et al., 2001) where the thick organic substance is reduced in fine and dissolved particles (Vannote et al., 1980). The decomposition process occurs in three distinct stages: i) leaching of soluble compounds, ii) microbial association and decomposition, and iii) fragmentation by macroinvertebrates (Abelho, 2001). The leaf decomposition is influenced by stream characteristics, as flow, pH (Webster and Benfield, 1986), water temperature (Irons et al., 1994), and nutrients (Gonçalves-Jr et al., 2006b). However, the chemical characteristics of the leaf litter are important factors since they would favor or not the association between microorganisms and invertebrates (Graça, 2001).

Graça et al. (2001) pointed out the importance of invertebrates in the leaf litter decomposition, mainly in lower order streams. The benthic fauna can accelerate the fragmentation process, facilitating the chemical compounds dissolution and the microorganisms colonization (Gonçalves-Jr et al., 2006b). Amongst the aquatic macroinvertebrates, Chironomidae (Diptera) is one of the most abundant groups in streams (Sanseverino and Nessimian, 2008), showing a great density and richness in leaf litter during decomposition (Gonçalves-Jr et al., 2003).

The abundance and composition of invertebrate communities are influenced by characteristics of vegetal species (Vannote et al., 1980) such as the physical structure, nutrients concentration and presence of secondary compounds as polyphenols and tannins (Graça, 2001). Hepp et al. (2009, in press) mentioned that the chemical complexity of *Eucalyptus grandis* Hill ex. Maiden leaves can influence the decomposition because some compounds may repeal the colonization of microorganisms and invertebrates. Graça et al. (2002) commented that in streams surrounded by *Eucalyptus globulus* Labill. plantations the invertebrates abundance was low, because the leaves of this plant were poor in energy for the organisms and showed great amount of essential oils and polyphenols.

In South of Brazil, species of *Eucalyptus* have been widely used, generating innumerable environmental problems and reducing areas with native vegetation, causing a reduction in regional biodiversity (Lima, 1996). *Eugenia uniflora* L. is a native species easily found in riparian regions of Rio Grande do Sul state. The present study had as aim to evaluate the structure and composition of the Chironomidae fauna associated with decomposing leaves of *Eucalyptus globulus* and *Eugenia uniflora*, observing if the larvae have colonization preferences for exotic or native species.

2. Material and Methods

2.1. Study area

The study was performed in a second-order stream located in Erechim, in the north of Rio Grande do Sul state (52° 14' 5.4" S and 27° 36' 43.5" W). The stream is located at 715 m a.s.l., the annual average temperature is 18 °C and annual average rainfall is 1,800 mm. The riparian vegetation is characteristic of Ombrophilous Alluvial Forest composed mainly by *Araucaria angustifolia* (Bert.) Kuntze, *Ocotea* spp., *Cabralea canjerana* (Vell.) Mart., *Eugenia uniflora* L. beyond the exotic species as *Eucalyptus* sp. and *Pinus* sp. (Trevisan and Hepp, 2007).

During the study, the waters presented a current velocity of 0.586 ± 0.232 $m.s^{-1}$. The average of water temperature was 15.7 ± 1.7 °C, whereas the average of conductivity was 56.4 ± 8.5 $\mu S.cm^{-1}$. The waters showed neutral pH, oscillating between acid lightly and alkaline lightly (6.99 ± 0.5) and were well oxygenated (8.25 ± 0.52 $mg.L^{-1}$). Precipitations occurred at the 21st and 25th day of the experiment (69.8 and 91.6 mm, respectively).

2.2. Field experiment

For the study senescent leaves of *Eucalyptus globulus* and *Eugenia uniflora were used*. The leaves were dry in temperature of 40 ± 2 °C until constant weight. The experiment was carried out between April and May of 2007. A total of 48 litter bags (dimensions 20 x 30 cm) with 2.0 mm of mesh size (Trevisan and Hepp, 2007) were incubated in riffle areas, with 3.0 ± 0.1g of leaves of each species. After 3, 6, 14, 21, 28, 35, 42 and 49 days of incubation, three litter bags of each vegetal species were removed randomly for the identification of the Chironomidae fauna and analysis of the leaves.

2.3. Vegetal debris

The vegetal material was collected and conditioned in plastic bags and ice. In laboratory, the leaf litter was gently washed for removal of the organisms and chemical analysis. For the determination of remaining mass and decomposition rates the leaves were oven-dried at 40 ± 2 °C until constant mass. The total polyphenols compounds of the vegetal leaves were determined by the Folin-Ciocalteu method in accordance with Bärlocher and Graça (2005).

2.4. Organisms identification

The Chironomidae larvae associated to the vegetal species were fixed in ethanol 80%. For the identification the larvae were cleared in KOH 50% for 24 hours and mounted slides with Hoyer medium. The larvae were identified at the genus level using an optic microscope under a magnification of 400X with the identification keys of Trivinho-Strixino and Strixino (1995) and Epler (2001). The classifications of the organisms in functional feeding groups (FFG) were made according to Merritt and Cummins (1996).

2.5. Data analysis

Breakdown rates were determined adjusting the values of leaf remaining dry mass to the negative exponential model $W_t = W_0.e^{-k}$, where Wt is the remaining mass at time t (in days), W_0 is the initial mass and k is the breakdown rate (Webster and Benfield, 1986). Analysis of covariance (ANCOVA) was used to test the differences in breakdown rates among leaf species (Gotelli and Ellison, 2004). The Chironomidae community was analyzed estimating organisms density (ind.g^{-1}DM), richness, determined by the number of identified genera, rarefied richness (Gotelli and Colwell, 2001) and the calculation of Shannon-Weaver Diversity index (Magurran, 2004). The differences in vegetal species and studied period were compared by Analysis of Variance (ANOVA two-way). The composition of Chironomidae fauna and the functional feeding groups associated to the leaf litter of both vegetal species were submitted to a Multivariate Analysis of Variance (MANOVA) to compare the groups of units by randomization tests with 1,000 permutations (Pillar and Orlóci, 1996), from matrices of Euclidean distance between units. To evaluate the differences between polyphenols of both vegetal species an Analysis of Variance was used (ANOVA two way). The total polyphenols was associated to the density of Chironomidae for the use of Linear Regression Analysis (Gotelli and Ellison, 2004). All statistical analyses were carried out with the software 5.0 BioEstat and Multiv (www.ufrgs.ecologia.br).

3. Results

After 49 days of the experimental period, the remaining dry mass of *Eugenia uniflora* leaves was slightly lower than that of *Eucalyptus globulus* (18.5 ± 15.7 and 19.9 ± 16.2%, respectively; Figure 1). The breakdown rate was slower in *E. uniflora* ($k = 0.036 \pm 0.026\ d^{-1}$) in relation to that of *E. globulus* ($k = 0.042 \pm 0.02\ d^{-1}$), being significantly different (ANCOVA, $F_{3,44} = 82.18$, $p < 0.0001$).

Fifteen Chironomidae genera of the subfamilies Tanypodinae, Chironominae and Orthocladiinae were identified (Table 1). *Polypedilum* Kieffer 1912, *Nanocladius* Kieffer 1912, *Thienemanniella* sp1 and *Thienemanniella* sp2 were found only in *Eucalyptus globulus* leaves, while *Pentaneura* Philippi 1865, *Lopescladius* Oliveira 1967, *Parametriocnemus* Goetghebuer 1932, *Paratendipes* Kieffer 1911, *Psectrocladius* Kieffer 1906, and Tanytarsini were exclusive of *Eugenia uniflora* leaves. There are no significant differences in the density of larvae between the leaves of both species ($p > 0.05$; Figure 3). *Rheotanytarsus* Thienemann and Bause in Bause, 1913 was present in both species, but appeared only after the sixth decomposition day (Table 1). This genus was the most abundant, with 105.97 ± 178.88 ind.g^{-1} leaf DM at the 42th decomposition day of *E. globulus*. In *E. uniflora*, the highest density was observed at the 49th day, with 58.67 ± 90.08 g^{-1} leaf DM. *Thienemanniella* sp3 was present in *E. globulus* between 14 and 42 days and in *E. uniflora* between 6 and 28 days of decomposition. The Chironomidae diversity and rarefied richness were not different between the vegetal species ($p > 0.05$), even so the richness was lightly superior in *E. uniflora* (Table 1, Figure 3). The variation in the Chironomidae community composition between *E. uniflora* and *E. globulus* was not significantly different (MANOVA, $p > 0.05$).

The organisms associated to the leaf litter were classified in three functional feeding groups, being the most frequent filtering-collectors, followed by the gathering-collectors and shredders (Figure 4). The great filtering-collectors abundance was because of the *Rheotanytarsus* density in both plants during the studied period. Gathering-collectors were represented by the majority of the identified genera (11 genera). The FFG composition showed no significant difference between the studied plants, but was significantly different among the studied days (MANOVA, $p = 0.35$ and $p = 0.001$, respectively).

The polyphenols contents oscillated between 3.87 and 4.61 ODU g^{-1}DM in *Eucalyptus globulus* and *Eugenia uniflora*, respectively (Figure 2). In *E. uniflora* the polyphenols were registered until day 14, whereas in *E. globulus* they were registered until day 28. The total polyphenols contents

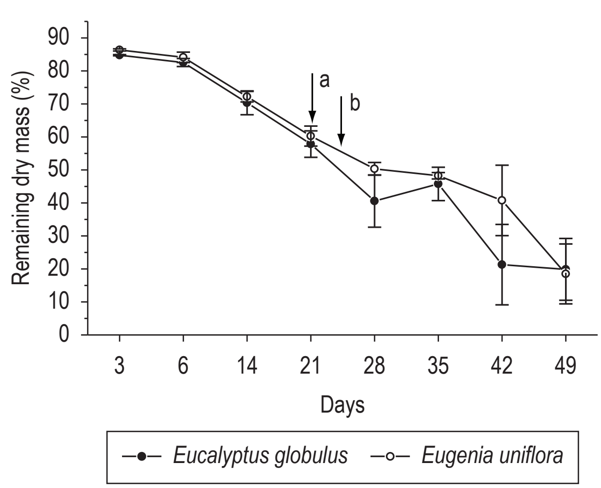

Figure 1. Leaf remaining dry mass (%) of *Eucalyptus globulus* and *Eugenia uniflora* in a Subtropical stream. The arrows indicate pluviometric events: a) 69.8 mm and b) 91.6 mm.

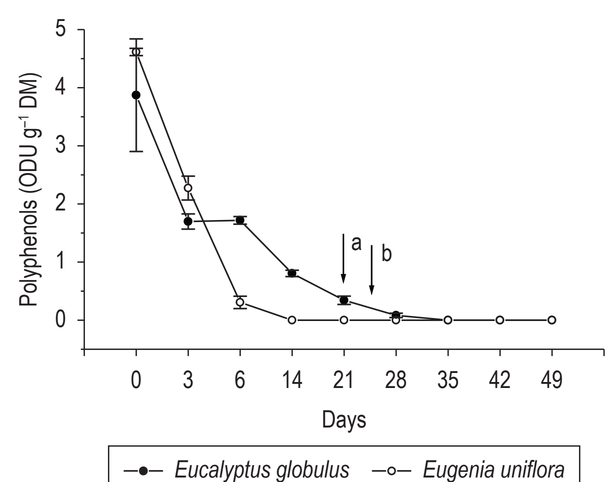

Figure 2. Polyphenols contents (ODU g^{-1}DM) of *Eucalyptus globulus* and *Eugenia uniflora* in a Subtropical stream. The arrows indicate pluviometric events: a) 69.8 mm and b) 91.6 mm.

Table 1. Chironomidae genera density (ind g[-1]DM). rarefied richness. and functional feeding groups (FFG: G-C: gathering-collectors; F-C: filtering-collectors; S: shredders) associated in *Eucalyptus globulus* and *Eugenia uniflora* leaves during the decomposition in Subtropical stream.

Genera	FFG	*Eucalyptus globulus*								*Eugenia uniflora*							
		Days															
		3	6	14	21	28	35	42	49	3	6	14	21	28	35	42	49
Chironominae																	
Paratendipes Kieffer, 1911	G-C	-	-	-	-	-	-	-	-	-	0.13	-	-	-	-	-	-
Polypedilum Kieffer, 1912	G-C/S	-	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rheotanytarsus Thienemann and Bause in Bause, 1913	F-C	-	2.06	6.85	24.10	15.49	3.77	105.9	8.41	-	1.56	4.75	10.53	7.31	1.86	13.58	58.67
Tanytarsini gen D	G-C	-	-	-	-	-	-	-	-	-	-	-	-	0.21	-	-	-
Tribelos Walker, 1848	G-C	-	-	-	-	-	-	-	1.10	-	-	-	-	-	0.23	-	-
Orthocladiinae																	
Cricotopus van der Vulp, 1874	G-C/S	-	-	-	0.18	-	0.22	0.51	-	-	0.13	-	-	-	0.24	-	-
Corynoneura Winnertz, 1846	G-C	-	-	0.17	0.18	0.35	-	-	-	-	0.26	-	-	-	-	-	-
Lopescladius Oliveira, 1967	G-C	-	-	-	-	-	-	-	-	-	-	0.15	-	0.24	-	-	-
Nanocladius Kieffer, 1912	G-C	-	-	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-
Parametriocnemus Goetghebuer, 1932	G-C	-	-	-	-	-	-	-	-	-	-	-	-	-	0.24	-	-
Psectrocladius Holmgren, 1869	G-C/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50
Thienemanniella Kieffer, 1911 *sp1*	G-C	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thienemanniella sp2	G-C	-	0.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thienemanniella sp3	G-C	-	-	2.79	4.08	6.14	0.22	10.93	-	-	0.13	1.06	2.37	0.66	-	-	-
Tanypodinae																	
Pentaneura Philippi, 1865	G-C	-	-	-	-	-	-	-	-	-	0.13	-	-	-	-	0.22	-
Rarefied Richness	-	1	2.47	2.34	1.99	2.15	2.47	2.65	2	0	4.31	2.21	1.91	2.26	4	1.22	1.58

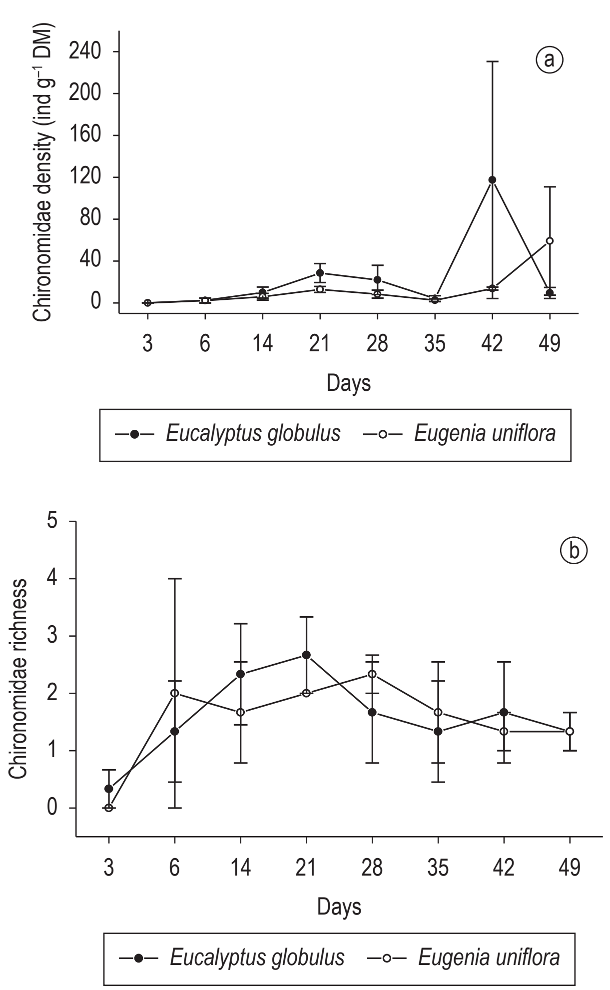

Figure 3. a) Density (ind g^{-1}DM) and b) richness of Chironomidae associated with *Eucalyptus globulus* and *Eugenia uniflora* in a Subtropical stream.

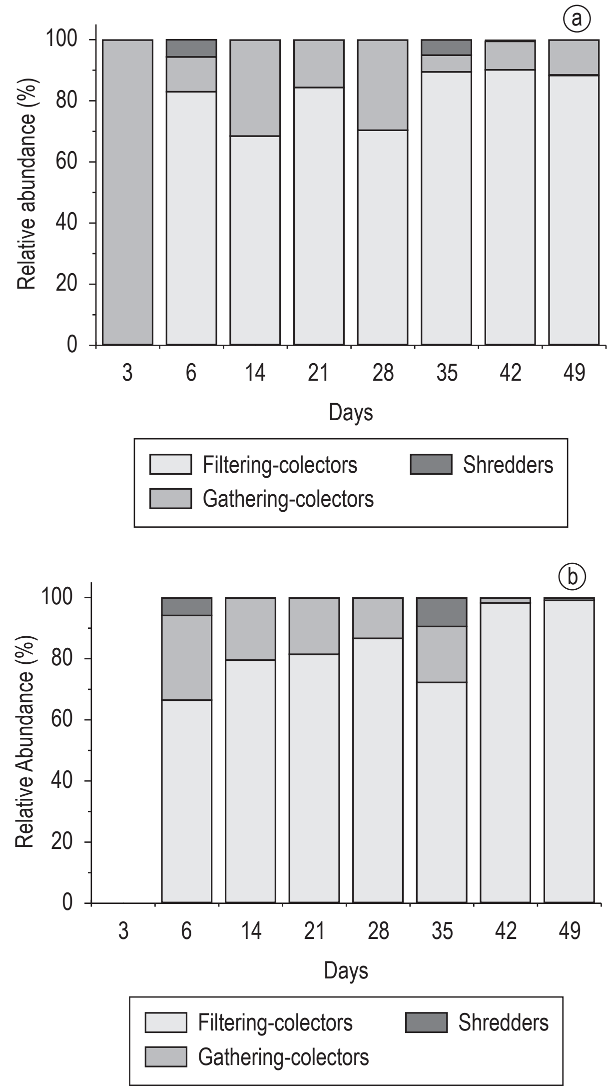

Figure 4. Relative abundance (%) of Chironomidae functional feeding groups associated with a) *Eucalyptus globules* and b) *Eugenia uniflora* in a Subtropical stream.

were not different between the two plants (2-ANOVA, $F_{1,25}$ = 1.78, p = 0.19), but showed variation during the studied days for both species (p < 0.0001). The influence of polyphenols contents on the density of Chironomidae larvae was significant for *E. globulus* and *E. uniflora* ($F_{1,23}$ = 4.77, p = 0.03 and $F_{1,23}$ = 8.65, p = 0.007, respectively).

4. Discussion

In the end of the studied period the dry mass remaining of both vegetal species were significantly different. Petersen and Cummins (1974) classified the breakdown rates as fast ($k > 0.01$ d^{-1}), intermediary (0.01 $d^{-1} > k > 0.005$ d^{-1}) and slow ($k < 0.005$ d^{-1}). In accordance with the results observed in this study, the decomposition of *Eucalyptus globulus* e *Eugenia uniflora* can be considered fast.

Trevisan and Hepp (2007), studying the decomposition of *Eucalyptus grandis*, observed that the species breakdown rates was slow ($k = 0.005$ d^{-1}). The authors attributed the result to the chemical composition of the species, which presents great variety of secondary chemical compounds. Hepp et al. (2009, in press) identified in *E. grandis* leaves a high variety of secondary compounds that possess allelopathics characteristics. Graça et al. (2002) pointed out that the decomposition of *Eucalyptus* leaves in streams is strongly influenced by the leaves chemical composition, which is rich in essential oils and polyphenols. However, the results of the present study suggest that the hydrologic characteristics of the stream contributed for the accelerated *Eucalyptus globulus* breakdown rates.

The rain occurrence in the days 21 and 25 of the experiment provoked an increase of the stream water flow, which was 0.4 $m.s^{-1}$ until day 20 but increased to 0.87 $m.s^{-1}$ between days 21 and 28. Gonçalves-Jr et al. (2006b), studying the decomposition rates of vegetal species in stretches of different orders in the same stream, observed a greater velocity of breakdown rates in higher order streams with higher current velocity, which increases the leaching process of the leaves compounds.

Chironomidae richness observed in the leaves of the studied species was similar to others studies carried out in tropical regions in Brazil. Gonçalves-Jr et al. (2000; 2003) observed 21 Chironomidae genera in leaves of *Typha dominguensis* Pers. and 9 in *Nymphaea ampla* L. in a coastal lagoon. Sanseverino and Nessimian (2008) found 26 genera in deposits of submerged leaves in a stream of the Atlantic Forest. Moretti et al. (2007) observed 24 genera associated to a different native species in Southeastern Brazil region. Jenke and Trivinho-Strixino (2007) found 16 genera in a tropical stream of low-order. Although the richness of the present study was similar to these studies, the fauna composition was very different. In the mentioned works *Polypedilum*, *Ablabesmyia* Johannsen 1905, *Asheum* Sublette 1964, and *Lauterborniella* Coquillett, 1902, were abundant genera. In the present study, *Rheotanytarsus* and *Thienemanniella* sp. 3 were most abundant and frequent during all the experiment period. This difference in the composition occurs because of the habitat type that, in the present study, was different in comparison to the others cited studies. *Rheotanytarsus* and *Thienemanniella* live in leaves fixed in rocks, preferentially in riffle areas. The litter bags in this case can be a stable environmental for these two genera, facilitating the colonization. *Rheotanytarsus* was a filtering-collector and the larvae construct homes with detritus in pipe form, fixed in rocks and leaves for the posterior part. In the anterior part they make nets to capture detritus particles. Roque et al. (2007) showed that the knowledge about Chironomidae fauna in the Neotropical region was limited, because some genera posses elevated numbers of similar species.

Sanseverino and Nessimian (2008) mentioned that some Chironominae genera occur in pools and when they appear in current waters they are associated to slow current in deposit zones, whereas Orthocladiinae is considered essentially as lotic organisms. Chironomidae larvae are dominant in the majority of aquatic environments possessing many food habits, even so few genera are considered shredders, this group can decisively influence the decomposition process (Moretti et al., 2007). The high densities of *Rheotanytarsus* and *Thienemanniella* indicated that these organisms can be associated to the leaf litter because of the fine particle in organic matter deposit or with erosion areas. Another reason that justified the density of *Rheotanytarsus* is that, for being filtering, they showed preference for places with riffles (Segura et al., 2007), corroborating the high density of this organism, mainly after day 20 when rains occurred. According to Graça (2001), the fragmentation by invertebrates can occur in direct or indirect way. The difference in the FFG composition in the leaf litter during the experiment period indicates that the Chironomidae association occurred independent to the vegetal species; however, the association was dependent of the study period, which presented hydrological variations caused by rains. Callisto et al. (2007) mentioned that some Chironomidae (not shredders) can use vegetal debris as a complementary food source.

The polyphenols contents in *Eucalyptus globulus* and *Eugenia uniflora* leaves showed a negative relation to the Chironomidae larvae colonization. The current velocity observed in the stream provoked a reduction of these leaves compounds after the day 21. The reduction of polyphenols contents facilitated the organisms association and contributed for the leaves breakdown. High current velocity provokes elevation in the leaching of chemical compounds of leaves as polyphenols, nitrogen and phosphorous (Trevisan and Hepp, 2007), facilitating leaf colonization by the organisms (Graça et al., 2002; Janke and Trivinho-Strixino, 2007).

The polyphenols showed repellent characteristics and reduced the palatability of the leaves. *Eucalyptus* possess great amount of essential oils and polyphenols that causes a slower leaf breakdown (Graça et al., 2002; Mason, 1980). Trevisan and Hepp (2007) observed that polyphenols showed a very important relation between leaf litter and invertebrates, mainly because of the great amount of these compounds in *Eucalyptus grandis* leaves, which presented direct relation among leaf breakdown, polyphenols contents and the invertebrates. Nitrogen and phosphorous contents and secondary compounds available in the plants are also important factors in the colonization of decomposing leaves. Hepp et al. (2009, in press) mentioned that these compounds were associated to the species defense methods against herbivorous and pathologic agents, what can interfere negatively in the decomposition process of the organic matter.

The absence of difference in the composition of Chironomidae fauna associated with the leaf breakdown of *Eucalyptus globulus* and *Eugenia uniflora* suggested that environmental factors have more importance for the leaf decomposition than the chemical differences between the two species. According to Graça et al. (2002), the *Eucalyptus* plantations affect the invertebrate communities mainly i) because the fungi colonization in leaves is low, which difficult the energy transference for shredders and ii) because leaves possess low nutritional quality and great amount of oils and polyphenols. The effects of exotic species on the aquatic biodiversity must be more studied. The low knowledge about the species chemical behavior during the decomposition process can influenced the microorganisms colonization and the benthic macroinvertebrates association and this influence make more difficult the energy flow in aquatic ecosystems.

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