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Effect of habitat heterogeneity in the composition and distribution of Chironomidae (Diptera) assemblage in different microhabitats of preserved streams in the Brazilian Atlantic Forest

Efeito da heterogeneidade de habitat na composição e distribuição da assembleia de Chironomidae (Diptera) em diferentes microhabitats de riachos preservados da Mata Atlântica Brasileira

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Abstract: Aim: This study aimed at to evaluate the influence of habitat heterogeneity on the attributes of richness, abundance, diversity and equitability of Chironomidae assemblage and also the biological differences in faunistic composition related to each microhabitat. **Methods:** The sampling was performed in summer (February) and winter (August) 2010, with Surber sampler, in three headwaters streams at Atlantic Forest, in southern Brazil. **Results:** In the total 6,429 Chironomidae larvae were identified and classified into 96 taxa belonging to Chironominae, Tanypodinae and Ortocladiinae subfamilies. Among the microhabitats, the highest richness was observed in the deposits of litter, and the highest number of exclusive species took place in the pool regions. **Conclusions:** Thus, the composition and structure of the Chironomidae assemblage are directly related to the availability and heterogeneity of habitats in streams.

Keywords: substrate, microhabitat, conservation unit, benthic invertebrates, richness.

Resumo: Objetivo: Este trabalho teve como objetivo avaliar a influência da heterogeneidade de habitats sobre os atributos de riqueza, abundância, diversidade e equitabilidade da assembleia de Chironomidae e também as diferenças biológicas na composição faunística relacionada a cada microhabitat. **Métodos:** As coletas foram realizadas no verão (fevereiro) e inverno (agosto) de 2010, com um amostrador tipo Surber. **Resultados:** As variáveis ambientais analisadas não foram significativamente diferentes entre os riachos amostrados, porém a riqueza taxonômica de larvas de Chironomidae foi distinta entre os microhabitats. Foram identificadas 6.469 larvas de Chironomidae distribuídas em 96 táxons pertencentes às subfamílias Chironominae, Tanypodinae e Ortocladiinae. Entre os microhabitats, a maior riqueza foi observada nos depósitos de folhiços em locais de remanso, onde também foram registrados os maiores números de espécies exclusivas. **Conclusões:** Portanto, a composição e estrutura da assembleia de Chironomidae estão diretamente relacionadas à heterogeneidade de habitats disponíveis nos riachos.

Palavras-chave: substrato, microhabitat, unidade de conservação, invertebrados bentônicos, riqueza.

1. Introduction

Benthic invertebrates are essential to the transformation of organic matter and nutrient cycling in aquatic environments (Graça, 2001; Devine and Vanni, 2002). The community represents an important component for the understanding of both structure and functioning of aquatic communities, as well as the ecological processes related to hydrological variations (Vannote et al., 1980). Several authors have studied the composition and distribution of benthic invertebrate assemblages searching for a better understanding of the natural structures of aquatic ecosystems and their biotic and abiotic relationships (Vannote et al., 1980; Ferrington-Junior, 1984; Rae, 2004; Inoue et al., 2005).

Among the benthic macroinvertebrates, the Chironomidae larvae is one the most abundant and diverse species in many aquatic environments, due to its adaptive strategy and diversity of feeding habits, making it an essential part of nutrient cycling within those ecosystems (Pinder, 1986; Cranston, 1995; Ferrington-Junior, 2008,). The distribution and composition of Chironomidae assemblages is related to the type of substrate, flow dynamics, water temperature and availability of organic matter of streams (Pinder, 1986; Berg, 1995; Sanseverino et al., 1998; Henriques-Oliveira et al., 2003; Amorim et al., 2004; Pinha, et al., 2013). Moreover, the group is affected by physical and chemical variables as a result of the different stages of organic matter decomposition, depending on both lotic site and microhabitat (Biasi et al., 2013).

There is a great substrate heterogeneity in headwater streams promoted by their source elements, such as allochthonous organic matter and rocks (Allan and Castillo, 2007). The substrate heterogeneity promotes a high diversity of microhabitats related to their distribution along the stream, to changes related to internal processes (cycling nutrients, moving bodies) and to external influences (Weatherproof, climatic and seasonal changes, etc.). In general, microhabitat diversity influences the invertebrate fauna, which increases habitat heterogeneity proportionally (Brown, 2007; Raio et al., 2011).

Related investigations show that a higher substrate heterogeneity and complexity increases richness and diversity of Chironomidae assemblage. Availability of shelter, protection, site for nidation along part or the entire life cycle and a rich epiphytic flora as well as other organic material for feeding provided by these environments were the main influencing factors determined by the authors (Callisto et al., 2007; Sanseverino and Nessimian, 2008; Silveira et al., 2013; König and Santos, 2013).

In this way, the present study aimed to evaluate the influence of habitat heterogeneity on the attributes of richness, density, diversity and evenness of Chironomidae assemblage and the biological differences in the faunistic composition related to each microhabitat.

Therefore, it was hypothesized that there will be an increase in richness, diversity and abundance of Chironomidae larvae attributes, especially in areas with rapids and in litter microhabitats. The amount of organic litter may offer more stability and anchorage places for the larvae, that together with stream flow increases the suspension food, also bringing nutrients and organisms. Further, due to the pristine characteristic of the environments we expect a higher quantity of rare or uncommon species in the microhabitat.

2. Material and Methods

2.1. Study area

The Iguaçu National Park (25°05' to 25°41' S and 53°40' to 54°38' W), located in the Western Paraná State, is a Conservation Unit that compounds one of the rare continuous and preserved areas of Atlantic Forest of interior, in Southern Brazil, considered to be one a biodiversity hotspot (Rylands and Brandow, 2005). The rivers, both entirely and partially protected by the boundaries of this unit (Figure 1), are references for the structure of aquatic ecosystem of this biome. The sampling region's climate is mild mesothermal and super humid, without droughts and potential occurrence of flooding throughout the year. Mean annual temperatures range between 18 and 20°C, with an absolute maximum between 34 to 36°C, and a minimum range of –8 to –4°C. The level of annual rainfall lies between 1,500 and 1,750mm.

2.2. Data collection

Sampling was conducted during summer (February) and winter (August) of 2010 using Surber sampler (0.04 m^2), with a mesh size of 200 μm in two sites of the three streams. At each site, triplicate substrate samples were taken from four different microhabitats for biological analyses, further one sample for granulometric texture analysis which resulted in 26 samples per stream.

The established microhabitats were denominated as followed: *litter pool* (PL, deposit of leaves at sites with little or no flow, near the banks), *litter riffle*

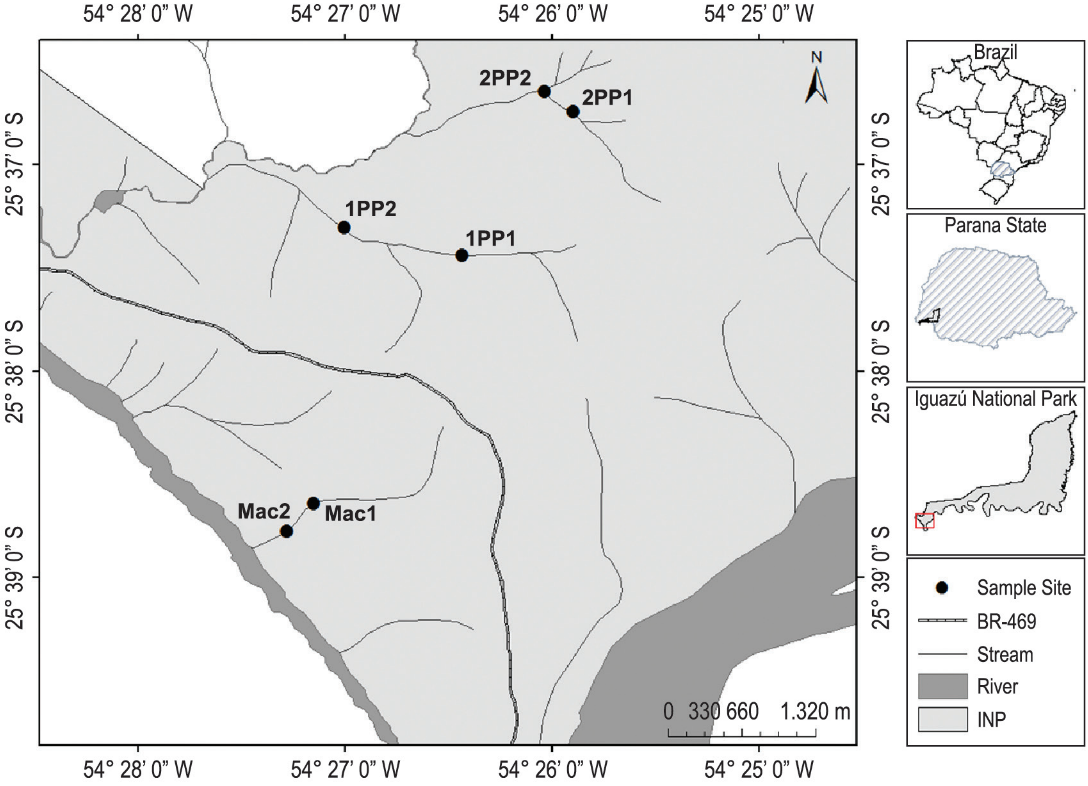

Figure 1. Location of the sampling sites in the Iguaçu National Park (INP). Mac1: stream of Macuco site 1; Mac2: stream of Macuco site 2; 1PP1: stream 1 of Poço Preto site 1; 1PP2: stream 1 of Poço Preto site 2; 2PP1: stream 2 of Poço Preto site 1; 2PP2A: stream 2 of Poço Preto site 2.

(RL, deposit of leaves retained in areas with rapids), *substrate pool* (PS, deposit of sediments at sites with little or no flow, near the banks) and *substrate riffle* (RS, sediments and material attached or deposited on rocks located in areas with rapids).

Concomitantly to sediment samplings, values of water temperature (°C), dissolved oxygen concentration (Microprocessor Oximeter AT 150), pH (pHmeter AT – 300), electric conductivity (portable conductivimeter), flow (m.s^{-1}) (river width x average depth x average speed of the flow), width (m) and depth (m) were measured.

After sampling, the sediment for biological analysis was cleaned under running water using a set of sieves with different mesh sizes (2.0, 1.0, and 0.2 mm). The material retained in the last mesh was placed in polyethylene bottles with 70% alcohol for further sorting under stereoscopic microscopes and identified to the lowest taxonomic level possible, with optical microscope (with increased of 400x and 1000x). The taxonomy was realized according to Epler (2001), Trivinho-Strixino and Strixino (1995) and Trivinho-Strixino (2011).

Sediment samples for granulometric texture analysis were oven dried at 80°C, grain texture was determined according to Wentworth scale described by Suguio (1973). The percentage of organic matter of the sediment was determined by the calcination of samples for 4 hours in a muffle furnace at 540°C, for determination of coarse particulate organic matter percentage (CPOM – particles > 1mm) and fine particulate organic matter (FPOM - particles < 1mm).

2.3. Statistical analysis

A Principal Component Analysis (PCA) was performed to summarize the physical, chemical and granulometric texture variables to ordinate possible groupings between the different seasons, sites or microhabitats of sampling. Using the scores of the PCA axes, a non-parametric analysis of variance (Kruskal-Wallis) was carried out to test the significance of the results, since the assumptions of homoscedasticity of variances had not been achieved.

The PCA axes were retained for interpretation according to Broken-stick criterion (the eigenvalues generated by the PCA should be higher than those generated at random in the Broken-stick and higher than three) (Gauch Junior, 1986; Jongman et al., 1995). In this study, only PC 1 was selected for interpretation, PC 2 was used only to construct the graph.

In order to describe the variations in the structure of Chironomidae larvae between the different sites, seasons and microhabitats, the following attributes were used: *i)* Density (Ind.m^{-2}), as an abundance measure, calculated per square meter using the area of the Surber sampler (0.04 m^2); *ii)* Shannon-Wiener diversity index (H'), described by the equation: $H' = -\sum(n_i/N).\log_2(n_i/N)$, where n_i is the number of individuals of the species *i* and *N*, the total number of individuals; *iii)* Evenness (E), obtained through the expression H'/H_{max}, where H_{max} is the diversity under maximum uniformity (Magurran, 1999) and *iv)* Taxonomic richness (S) – number of registered taxa. The differences between the values of these attributes for seasons, sites and microhabitats were tested by running a factorial ANOVA considering the significance of $p < 0.05$.

The interaction between the Chironomidae assemblages in the different microhabitats was verified through the Jaccard coefficient that indicates similarity between sites considering the species composition (McCune and Mefford, 2002).

The similarity in the abundance of the benthic invertebrate community in the streams was examined using a non-metric multidimensional scaling (NMDS) being one of the most robust ordination analyses to nonlinear situations (Clarke, 1993). The distances were calculated through the Bray-Curtis similarity index. For the number of dimensions generated we choice k = 2. The resolution distortion in two dimensions is expressed by the value of *S* (*stress*). The closer to zero the *stress* is, the better the fit between the original distance of the objects and the arrangement obtained by the analysis (Legendre and Legendre, 1998).

Based on the density of Chironomidae larvae population, a Detrended Correspondence Analysis (DCA) was performed to reduce biotic data dimensionality and ordinate the microhabitats. The most significant taxa were correlated with the axes 1 and 2 of the DCA through the Pearson Correlation Analysis (McCune and Mefford, 2002).

For the accomplishment of the multivariate analyses PCA and DCA, data was previously logarithmized (log x+1) and transformed $(b=Xi, j)^p$, with p=0.5, respectively, to minimize the effect of outliers.

The multivariate analyses (PCA and DCA), the NMDS, the Jaccard coefficient and the values of Shannon-Wiener diversity and evenness were carried out in the software PC-ORD version 5.0 (McCune and Mefford, 2002). The analysis of variance and Pearson correlation as well as the graphs were done using Statistica 7.1 (2005).

3. Results

3.1. Physical and chemical characterization of the sampling sites

No marked and statistically significant differences were detected (p = 0.4) in the values of physical and chemical water variables, between the different streams and their respective sampling sites and seasons (Table 1).

Both PC 1 and PC2 added up to 47.6% of explanation of variability of environmental data analyzed (Table 1). However, no grouping pattern statistically significant was identified for the streams, sites or seasons through the PCA (Figure 2a).

The scores 1 and 2 of the principal components did not provide differentiated groups, indicating that the environmental variables did not follow trends or patterns for the streams, sites and seasons. The PC 1 of PCA was negatively influenced by the percentage of coarser sand and higher flow, though positively by the percentage of medium, fine and very fine sand (Figure 2b).

The granulometric texture in the different sites and streams was statistically similar, with substrate predominantly composed of pebbles, granules and very coarse sand. In these environments, in general, the coarse organic matter occurred at higher percentage than the fine organic matter (Figure 2a, b).

3.2. Chironomidae Assemblage

A total of 6,469 Chironomidae larvae were collected, totaling 96 taxa belonging to the subfamilies Chironominae (44 taxa), Tanypodinae (23 taxa) and Ortocladiinae (19 taxa). While analyzing the density, richness, evenness and diversity (Shannon-Wiener diversity index (H')) of assemblage in relation to season, site and microhabitat, significant differences were recorded

Table 1. Eigenvalues of the Principal Component Analysis by the Broken-stick criterion, percentage of explanation of the axes, and Kruskal-Wallis analysis of variance (KW). P: significance.

Axis	Eingenvalues	% of Variance	% Acumulation	Broken-stick	KW-H (11,12)
1	5.54	32.588	32.588	3.44	p=0.4
2	2.555	15.029	47.617	2.44	p=0.4

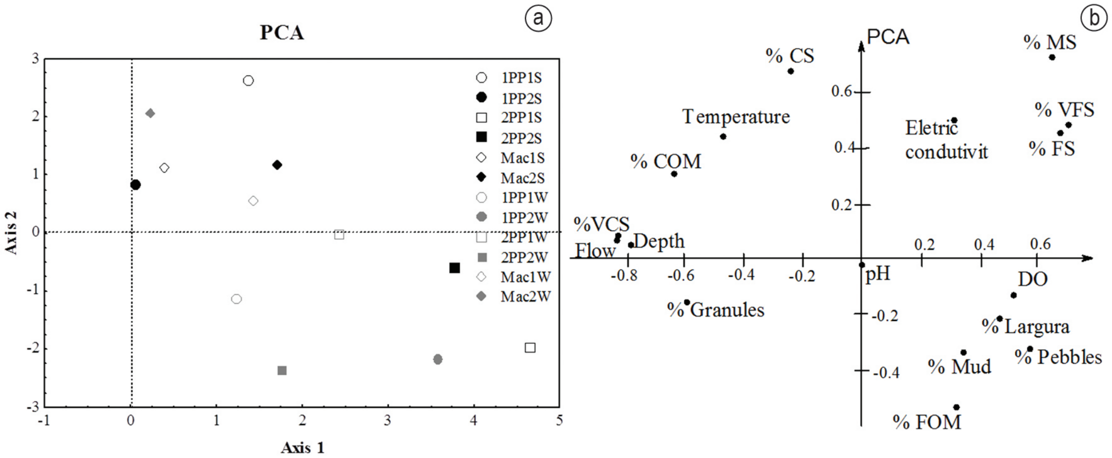

Figure 2. Scores ordination of the PC 1 and 2 of PCA for the sampled streams (a) and the vectors of the variables physical, chemical and of grain texture with the score of the PC 1 and 2 of PCA (b). 1PP1S: stream 1 of Poço Preto site 1, Summer; 1PP2S: stream 1 of Poço Preto site 2, Summer; 2PP1S: stream 2 of Poço Preto site 1, Summer; 2PP2S: stream 2 of Poço Preto site 2, Summer; Mac1S: stream of Macuco site 1, Summer; Mac2S: stream of Macuco site 2, Summer; 1PP1W: stream 1 of Poço Preto site 1, Winter; 1PP2W: stream 1 of Poço Preto site 2, Winter; site 2; 2PP1W: stream 2 of Poço Preto site 1, Winter; 2PP2W: stream 2 of Poço Preto site 2, Winter; Mac1W: stream of Macuco site 1, Winter; Mac2W: stream of Macuco site 2, Winter; VFS = very fine sand; FS = fine sand; MS = medium sand; CS= coarse sand; VCS = very coarse sand, COM: coarse organic matter, FOM: fine organic matter.

in larval distribution for the four observed microhabitats (Figures 3a to 3i).

Among the microhabitats, higher density, richness and diversity of Chironomidae larvae occurred in the deposits of litter, both in the riffle (RL) and pool (PL). The highest evenness was observed for the taxa collected in the substrate of the pool (PS) and riffle (RS).

In the latter, lower values of richness and diversity were recorded (Figure 3g, h, i).

Despite that densities and richness observed in different sampling sites had not presented significant differences, increase in these biological attributes in site 2 in relation to site 1, especially in the stream 2 of Poço Preto and Macuco was notable (Figure 3a, b, c).

3.3. Microhabitats

With regard to microhabitats, the highest richness was observed in the deposit of litter in pool and riffle, with 81% and 72% of the total registered taxa, respectively. In the substrate of pool and riffle, 65% and 49%, respectively, of the total Chironomidae richness was recorded (Table 2). For richness, mean densities were higher in the litter pool and riffle. In the areas with riffle, LR and SR, the greatest density was of *Rheotanytarsus,* whereas *Caladomyia* sp.5 was abundant in the litter pool, and *Polypedilum* (*Tripodura*)*,* in the substrate pool (Table 2).

Of the total of 96 taxa registered, 33 were commonly found in the four microhabitats, distributed among eleven genera of the subfamilies Chironominae (*Caladomyia*, *Lauterborniella*, *Oukuriella*, *Polypedilum*, *Pseudochironominae*, *Rheotanytarusus*, *Stempellinella*, *Stenochironomus*, *Tanitarsinii*, *Tanytarsus* and *Zavreliella*), six representatives of the subfamily Ortocladiinae (*Corynoneura*, *Cricotopus*, *Lopescladius*, *Nanocladius*, *Onconeura* and *Parametriocnemus*) and four belonging to the subfamily Tanypodinae (*Ablabesmyia (Karelia)*, *Hudsonimyia*, *Larsia* and *Djamabatista*).

A high overlap was also registered among the litter pool (PL) microhabitats, litter riffle (RL) and substrate pool (PS) with 15 morphospecies represented by *Cardiocladius* sp., *Clinotanypus* sp., *Criptochironomus* sp.2, *Denopelopia* sp., *Endotribelos* sp.1, sp.3 and sp.4, *Labrundinia* sp.1, *Polypedilum* sp.2, *Parapentaneura* sp.1, *Pentaneura* sp., *Stempellina* sp., *Tanypodinae* sp., *Xestochironomus* sp. and *Zavreliella* sp1.

The grouping analysis of Jaccard also indicated high similarity between microhabitats PL, RL and PS, differing from the substrate riffle (RS). Among them, the highest number of exclusive species was within the litter pool microhabitat (12) followed by the substrate pool (6), litter riffle (4) and substrate riffle with only one species (Figures 4a, b).

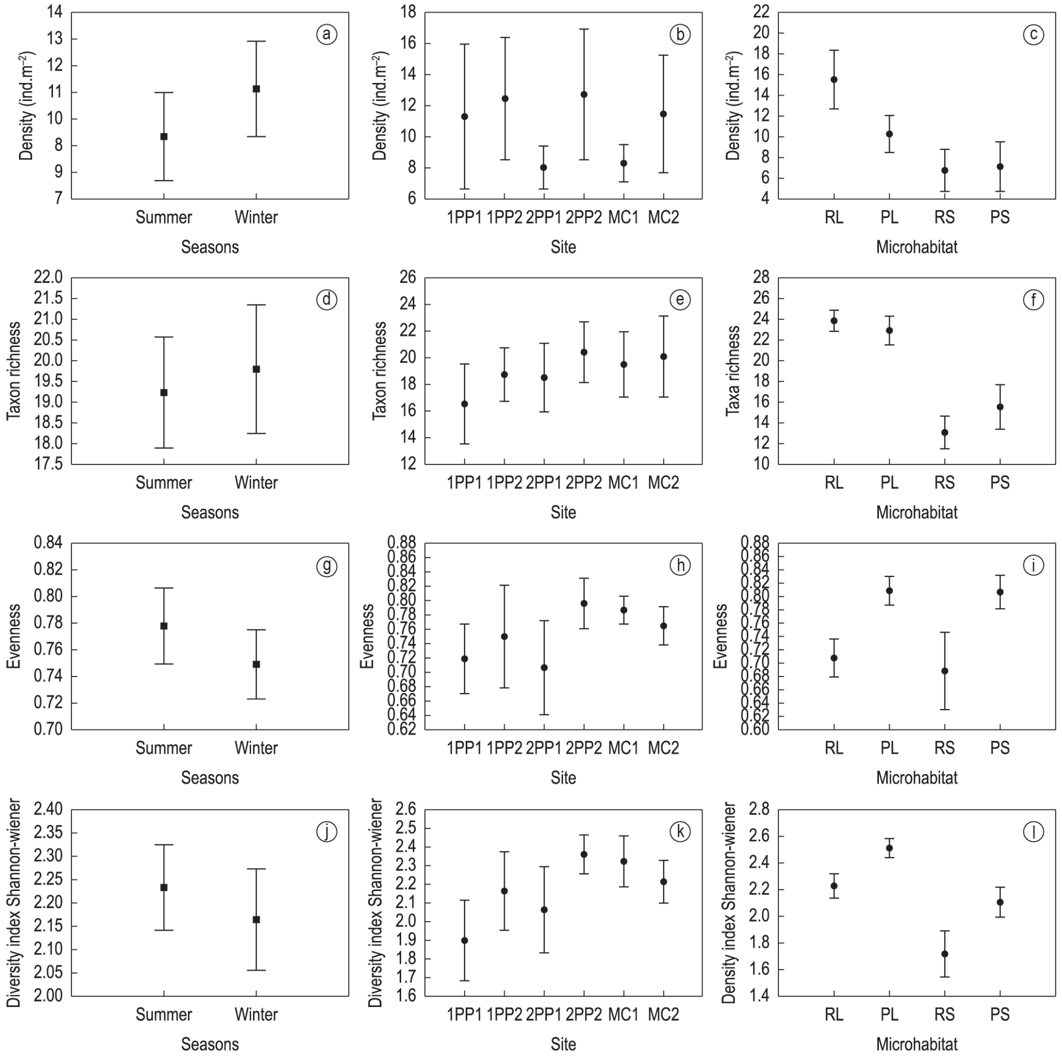

Figure 3. Mean density (a, b, c), richness (d, e, f), evenness (g, h, i) and diversity (j, k, l) of Chironomidae assemblage per seasons, sites and microhabitats. 1PP1: stream 1 of Poço Preto site 1, 1PP2: stream 1 of Poço Preto site 2, 2PP1: stream 2 of Poço Preto site 1, 2PP2: stream 2 of Poço Preto site 2, MC1: stream Macuco site 1, MC2: stream Macuco site 2. FC: riffle litter, FR: pool litter; SC: riffle sediment, SR: pool sediment.

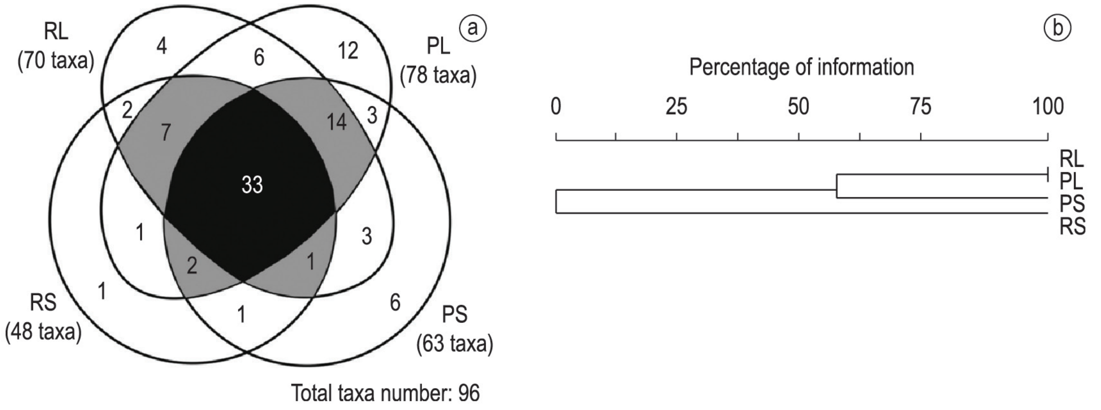

Figure 4. Schematic diagram of the distribution (a) and Grouping analysis by Jaccard Coefficient (b) of the Chironomidae taxa per microhabitats sampled. RL: riffle litter, PL: pool litter, RS: riffle sediment, PS: pool sediment.

Table 2. Occurrence and distribution of Chironomidae taxa (presence indicated by gray cells) and their respective densities in the different microhabitats. Where: 0-50 ind.m^{-2}:*, 50-100 ind.m^{-2}:**, 100-200 ind.m^{-2}:***, 200-300 ind.m^{-2}:**** and 300-500 ind.m^{-2}:*****. RL: riffle litter; PL: pool litter; RS: sediment riffle; PS: sediment pool.

Taxon	RL	PL	PS	RS	Taxon	RL	PL	RS	PS	Taxon	RL	PL	RS	PS
Ablabesmyia	*	**	*	*	*Labrundinia* sp.2	*	*	*		*Thienemanniella* sp.2	*		*	
Caladomyia sp.5	*	***	*	**	*Nilotanypus*	*	*	*		Ortocladinae sp.	*			*
Caladomyia sp.8	*	*	*	*	Ortocladinae B	*	*	*		*Paramétriocnemus* sp.2	*			*
Caladomyia sp.9	*	*	*	*	*Polipedilum* sp.1	*	**	*		*Paraphaenocladius*	*			*
Caladomyia sp.10	*	*	*	*	*Pseudochironomus* sp.2	*	*	*		*Nilothauma* sp.3		*	*	
Caladomyia sp.4	*	*	*	*	*Riethia*	*	*	*		*Cryptochironomus* sp.1		*		*
Corynoneura sp.2	**	*	*	*	*Thienemanniella* sp.1	****	*	*		*Labrundinia* sp.3		*		*
Corynoneura sp.3	***	*	*	*	*Cardiocladius*	*	*		*	*Pentaneurini*		*		*
Cricotopus sp.1	*	*	*	*	*Clinotanypus*	*	*		*	*Labrundinia* sp.10			*	*
Cricotopus sp.2	*	*	*	*	*Cryptochironomus* sp.2	*	*		*	*Beardius* sp.2	*			
Cricotopus sp.3	*	*	*	*	*Denopelopia*	*	*		*	*Metriocnemus*	*			
Djalmabatista sp.2	*	*	*	**	*Endotribelos* sp.1	*	**		*	*Parapentaneura* sp.2	*			
Hudsonimyia	*	**	*	*	*Endotribelos* sp.3	*	*		*	*Rheocricotopus*	*			
Larsia	*	*	*	**	*Endotribelos* sp.4	*	*		*	*Antillocladius* sp.1		*		
Lauterborniella	*	*	*	*	*Labrundinia* sp.1	*	*		*	*Caladomyia* sp.7		*		
Lopescladius sp.1	*	*	*	*	*Polipedilum* sp.2	*	*		*	*Chironomus fittkaui*		*		
Lopescladius sp.2	*	*	**	*	*Parapentaneura* sp.1	*	*		*	*Dicrotendipes sp.3*		*		
Nanocladius	***	**	*	*	*Pentaneura*	*	*		*	*Dicrotendipes* sp.3		*		
Onconeura	****	*	*	*	*Stempellinella* sp.	*	*		*	*Endotribelos* sp.5		*		
Oukuriella	*	*	*	*	Tanypodinae sp.	*	*		*	*Fittkauimyia* sp.1		*		
Polipedilum(Tripodura)	*	***	*	****	*Xestochironomus*	*	*		*	*Fittkauimyia* sp.2		*		
Polipedilum gr. *Fallax*	*	*	*	*	*Zavreliella* sp.1	*	*		*	*Labrundinia* sp.4		*		
Parametriocnemus sp.1	**	*	*	*	*Labrundinia* sp.8		*	*	*	*Labrundinia* sp.7		*		
Pseudochironomus sp.1	*	*	*	*	Tanytarsini sp.B		*	*	*	*Labrundinia* sp.11		*		
Rheotanytarsus	*****	*	*****	*	*Caladomyia* sp.11	*		*	*	*Procladius*		*		
Stempellinella sp.1	*	*	*	*	*Antillocladius* sp.2	*	*			*Stempellina*			*	
Stempellinella sp.2	*	*	*	**	*Beardius* sp.1	*	*			*Caladomyia* sp.6				*
Stenochironomus	*	*	*	*	*Corynoneura* sp.1	*	*			*Coelotanypus* sp.3				*
Tanytarsini A	*	*	*	*	*Endotribelos* sp.2	*	*			*Labrundinia* sp.				*
Tanytarsus B	*	*	*	**	*Endotribelos* sp.6	*	*			*Paralauterborniella*				*
Tanytarsus rhabdomantis	**	*	*	*	*Nilothauma* sp.1	*	*			*Procadius* sp.3				*
Zavreliella sp.2	*	*	*	*	*Paratendipes*		*			Tanytarsini sp.				*

The NMDS evidenced a value of *stress* around 0.04 and p = 0.04 (Monte Carlo Test), ensuring a good reliability in interpreting the abundance results. This analysis pointed out a greater similarity among the microhabitats of riffle (evidenced can be found in Figure 5) considering the overlap of the closed points. The litter pools were more similar to each other (points more grouped) than the substrate rapids (points more disperse). On the other hand, the microhabitats of pool were less similar to each other, and in relation to the riffle (more spread points) (Figure 5).

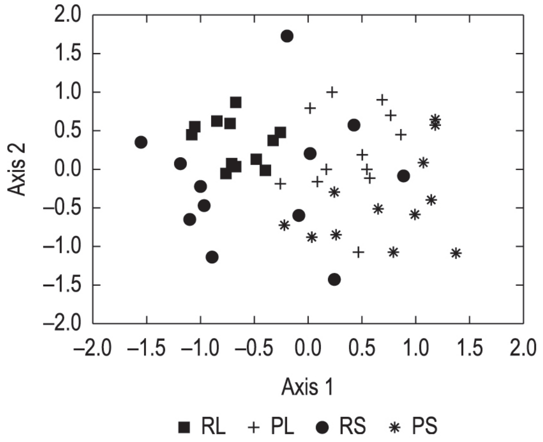

Figure 5. Non-metric multidimensional scaling (NMDS) for the density of benthic invertebrates in the microhabitats of the streams of the Iguaçu National Park. RL: riffle litter, PL: pool litter, RS: riffle sediment, PS: pool sediment.

The results of the DCA, based on the densities of taxa registered for the different microhabitats, presented eigenvalues of 0.26 for the axis 1 and 0.15 for axis 2. The scores distribution indicated a trend in the distribution of the different taxa, in relation to the microhabitats of riffle and pool, regardless to substrate or presence of litter (Figure 6a).

The Pearson correlation performed between the scores of the DCA axes and the analyzed taxa indicated that *Caladomyia* sp.5*, Stempellinella* sp.2, *Polypedilum* (*Tripodura*) and *Djalmabatista* sp.2 have presented a highly positive correlation for axis 1, while for axis 2, correlation was observed for *Lopescladius* sp.2. Negatively, axis 1 had strong correlation with *Rheotanytarsus*, *Onconeura* and *Parametriocnemus* sp.2, and axis 2, with *Fittkauimyia* sp.1 (Figure 6b).

4. Discussion

The results pointed out physical, chemical granulometric texture similarities of all three streams. In the same way, there were no significant differences within biological attributes (richness, Shannon diversity index, evenness and density) in relation to season and different sampling sites, indicating that environmental variables exerted no influence on the composition of different Chironomidae taxa.

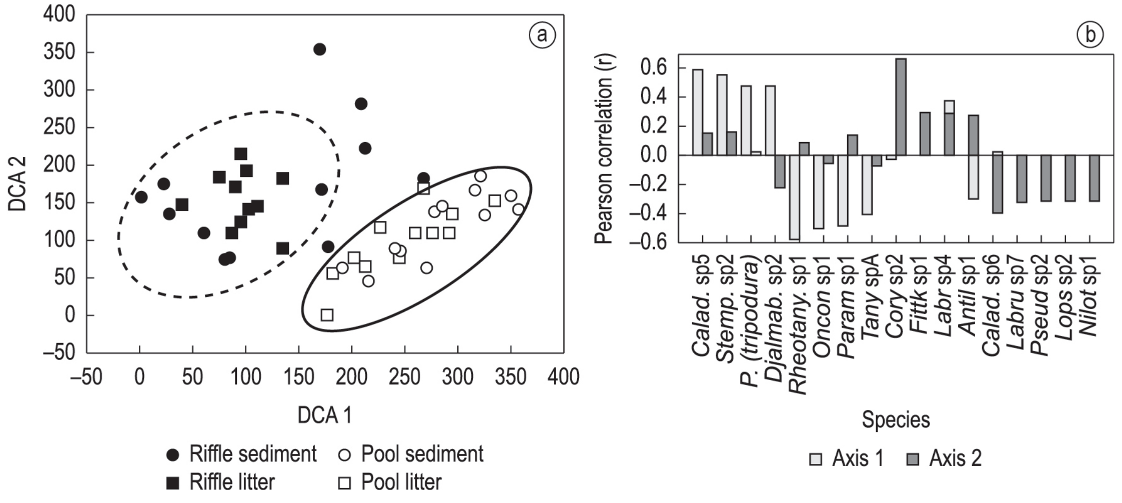

Figure 6. Scores ordination of the axes 1 and 2 of DCA per microhabitats sampled (a) and Pearson correlation between the most significant taxa with the scores of the axes 1 and 2 of DCA (b). *Calad.* Sp5: *Caladomyia* sp.5; Stemp.sp2: *Stempellinela* sp.2; *P.(Tripodura)*: *Polypedilum* (*Tripodura*); *Djalmab* sp2: *Djalmabatista* sp.2; *Rheotany.* sp1: *Rheotanytarsus* sp1; *Oncon* sp1: *Onconeura*; *Param* sp1: *Parametriocnemus* sp.1: *Tany* spA: *Tanytarsini* sp.A; *Cory* sp2: *Corynoneura* sp.2; *Fittk* sp1: *Fittkauimyia* sp.1; *Labr* sp4: *Labrundinia* sp.4; *Antil* sp1: *Antillocladius* sp.1; *Calad.* sp6: *Caladomyia* sp.6; *Labrun* sp7: Labrundinia sp.7; *Pseud* sp2: *Pseudochironomus sp.*2; *Lops* sp2: *Lopescladius* sp.2; *Nilot* sp1: *Nilotanypus* sp.1.

However, these same biological attributes were significantly different when compared to the four microhabitats. Thus, among the different variables, the heterogeneity of environments was crucial to the occurrence of different colonization patterns of the assembly of chironomids, as initially supposed in our hypothesis.

The high environmental integrity and low-order streams may have contributed to the increased heterogeneity of microhabitats and therefore favored the high richness and diversity of larvae of this family. Since the large contribution of allochthonous material source from riparian vegetation is favorable to colonization of aquatic invertebrates (Allan and Castillo, 2007; Callisto et al., 2007; Moretti et al., 2007; Sanseverino and Nessimian, 2008; Rosin et al., 2010). However, this fact is also a controversy topic, because some studies have demonstrated that the richness tend to be lower in headwater streams and increase with the stream order (Clarke et al., 2008), although the contribution of these smaller streams for the local richness (Grönroos and Heino, 2012) and regional diversity becomes apparent only at larger scales (Clarke et al., 2008). Nevertheless, the high taxa richness in our study, considering only 3 streams and two sampling periods, demonstrates the importance of these preserved environments for the maintenance of the group, especially when compared to the Chironomidae richness from other regions worldwide (Ferrington-Junior, 2008).

A lot of studies have emphasize the positive relationship and importance between the presence and quality of riparian vegetation both to Chironomidae and other invertebrates (Callisto et al., 2002; Kleine and Trivinho-Strixino, 2005; Callisto et al., 2007; Hepp et al., 2008; Ligeiro et al., 2013; Bruder et al., 2014). The vegetal cover also provides greater stability in terms of flow and consequently of community structure (Suriano and Fonseca-Gessner, 2004) that contribute to the habitat heterogeneity ensuring an assemblage with high richness, composed of genera and species with diversified ecological requirements and widely distributed (Amorim et al., 2004; Brown, 2007; Kon and Korukura, 2011), as found in the present study.

The high richness and diversity is related to the high number of taxa uncommon in other types of environments such as *Hudsonimyia, Parametriocnemus, Cardiocladius, Antillocladius, Orthocladiinae* sp., *Paraphaenocladius, Metriocnemus, Fittkauimyia;* or specialist taxa as *Stenochironomus* e *Xestochironomus,* commonly associated with woody substrates and *Oukuriella*, genus inhabiting freshwater sponges (Trivinho-Strixino, 2011). The higher richness and uncommon taxa can be due to the environmental conservation of the sites that favors the maintenance of the microhabitats heterogeneity and consequently promotes better conditions of colonization to the Chironomidae assemblage (Suriano and Fonseca-Gessner, 2004; Siqueira and Trivinho-Strixino, 2005).

The number of rare species is a direct function of the richness: the more species an assemblage, the more species are classified as rare. This statement explains the fact that a higher number of rare species was found in the richest substrate (PL), and a lower number of rare species in substrates of lower richness (RS). Furthermore, although the definitions of rarity are necessarily arbitrary, there is a consensus that a species may be rare or common in relation to its number or distribution, and, despite some exceptions, the most widely distributed species tend to occur in higher abundance (Gaston, 1994).

Within microhabitats, Chironomidae larvae with varied ecological requirements were found as well despite the fact that specialized taxa usually is restricted to a single type of substrate, (such as *Stempellinella* and Tanytarsini, being characteristical for sandy bottom, or, *Beardius* and *Zavreliella* associated with vegetal fragments), to those genera commonly found in different lotic ecosystems, such as *Larsia*, *Ablabesmyia (Karelia)*, *Cricotopus*, *Onconeura* and *Parametriochinemus* (Trivinho-Strixino, 2011).

The initial hypothesis of this study that in microhabitat rapid litter (RL), attributes of the communities would be higher was not supported. We expected that in fast waters, the specimens would use the habitat conditions to displacement and getting food, and that the presence of plant fragments arrested the currents would provide stability, and consequently conditions to harbor a greater variety of taxa with diverse ecological needs (Coffman and Ferrington-Junior, 1984) however, most taxa preferred habitats compounded of litter in pool areas (PL).

Since the slower water speed facilitates the colonization and permanence of the specimens, and increased the feed supply by the accumulation of organic detritus, as wood, leaves, particulate material and even periphyton present in higher amount (Coffman and Ferrington-Junior, 1984; Callisto et al., 2007; König and Santos, 2013). In this microhabitat we can highlight *Stenochironomus*, *Endotribelos* and *Cryptochrironomus*, can be highlighted being exclusively xylophages (miner wood) and therefore reflecting the high availability of food from riparian vegetation (Siqueira and Trivinho-Strixino, 2005). Besides being a source of food, pool areas also provided shelter and is used to build tubes by tubificid larvae such as *Lauterboniella*, *Corinoneura*, and *Nanocladius* as observed in the literature (Hirabayashi and Wotton, 1999; Sanseverino and Nessimian, 2008).

In this way, pool litter (PL) microhabitat includes features like the availability of organic matter coupled to stability derived from the slow flow, thereby favoring colonization and permanence of a wide variety of taxa. This contributed to a higher richness and density of Chironomidae larvae as well. Within pool microhabitats, the highest number of unique morphotypes as *Antillocladius*, *Caladomyia*, *Fitkauimyia*, *Procladius* and *Dicrotendipes* species, know to demand higher environmental requirements, were found (Larnon and Carter, 2000). The dominant genus was *Caladomyia*, commonly associated with shallow sediments and *Polypedilum* (*Tripodura*) frequently found in different lotic and lentic environments (Trivinho-Strixino, 2011; Sanseverino and Nessimian, 2008).

On the other hand, in pool substrates (PS) without deposit of organic matter, restricting the availability of food, we noticed a large reduction in number of biological attributes, probably caused by the low availability and variety of food.

However, lower density, diversity and richness were recorded in rapid substrate (RS) environments characterized by greater instability. This indicates that in the absence of particles to attach, the larvae are exposed to the strength of the current, causing that in the habitats of fast water the colonization is realized by species having specific adaptations for fixation such as two dominant species at this site: *Rheotanytarsus,* (larvae constructors of tubes) that feed on particles brought by the current (Henriques-Oliveira et al., 2003) and *Lopescladius*, also a tube constructor, commonly found in sand substrates streams (Sanseverino and Nessimian, 2001). These environmental requirements may explain why the taxa grouped in this microhabitat are of such difference when compared to those occurred in the other groups.

Moreover, in both pool microhabitats (PL, PS) a higher evenness was found, indicating that in these sites the taxonomic abundance was similar, including the high density of *Polypedilum* (*Tripodura*) and *Caladomyia* sp.5 larvae. According to Roque et al. (2000) and Fonseca-Leal et al. (2004) *Caladomyia* (Tanytarsini Tribe), are predominant in natural areas and related with environmental conditions characteristic of less impacted areas. On the other hand, *Polypedilum (Tripodura)* represents one of the most diverse and widespread species group (Trivinho-Strixino, 2011).

Nevertheless, the composition and structure of Chironomidae assemblage were directly related to the availability and type of substrate of streams. These results emphasize the importance of habitat heterogeneity and of flow dynamics for benthic communities (Sanseverino et al., 1998; Henriques-Oliveira et al., 2003).

Furthermore the microhabitats of higher preference for Chironomidae assembly were pool litters followed by riffle litters, highlighting how the deposits and fragments of leaves in streams are essential to ensure colonization in greater diversity, especially in relation to microenvironments exposed to current.

Our understanding of the conservation status of both species and ecosystem is poor worldwide (MacNally and Fleishman, 2004). However, streams in fully preserved areas to assess the distribution of biological communities are increasingly scarce (Tupinambás et al., 2007). Thus, assess their current state and estimating how environmental characteristics may affect their future condition are vital measures for making decisions that maximize protection of biodiversity and the services provided by the ecosystem. As demonstrated, environmental heterogeneity can be linked to how rich an environment will be in number of taxa of Chironomidae, thus contributing to future studies on composition and richness of this community. Ensuring habitats heterogeneity is essential to maintaining the diversity of Chironomidae, considering that the areas of greatest preference for colonization of the assembly are in locations of materials deposition.

Therefore, environmental preservation areas contribute decisively to the integrity of aquatic ecosystems, reflected by the community attributes, besides the high ecological diversity favored by the maintenance of structural patterns and habitat heterogeneity. Despite their importance, few studies had been performed with ecological and taxonomical focus on aquatic invertebrates in conservation units.

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