ZOOLOGIA 32 (3): 207–214, June 2015
http://dx.doi.org/10.1590/S1984-46702015000300005

Intra- and inter-annual variations in Chironomidae (Insecta: Diptera) communities in subtropical streams

Diane Nava[1], **Rozane M. Restello**[1] & **Luiz U. Hepp**[1,*]

[1] *Programa de Pós-graduação em Ecologia, Universidade Regional Integrada do Alto Uruguai e das Missões. Avenida Sete de Setembro 1621, 99709-910 Erechim, RS, Brazil.*
**Corresponding author. E-mail: luizuhepp@gmail.com*

ABSTRACT. The structure and composition of stream benthic communities are strongly influenced by spatial and temporal factors. This study evaluated the intra and inter-annual variations in Chironomidae communities in subtropical streams. The organisms were sampled from 10 small-order streams during the summer and winter of 2010-2012. The number of chironomid specimens sampled was 7,568, distributed in 49 genera. Chironomid abundance and richness varied intra and inter-annually and community composition varied intra-annually (2010 and 2011). Water temperature, total organic carbon, nitrogen, and rainfall were correlated with chironomid community composition. The intra-annual variation of the community was dependent on climatic variations (temperature and rainfall) and changes caused by intensive agricultural use. We conclude that the temporal variation observed in the Chironomidae community correlates with climatic variations (rainfall) and changes in the total organic carbon and total nitrogen, caused by intensive agricultural land use.

KEY WORDS. Agriculture impacts; bioindicators; macroinvertebrates; rainfall.

The structure and composition of aquatic communities are influenced by spatial and temporal factors (SUAREZ 2008). Knowing how these factors act on biological communities facilitates the understanding of how local and regional factors influence species occurrence (POFF et al. 2006, SUAREZ 2008). The distribution of benthic macroinvertebrates is affected by factors such as type of substrate (HEPP et al. 2012), habitat characteristics (GALDEAN et al. 2000, BUSS et al. 2004), land use (HEPP et al. 2010), and climatic variations over a timescale (SCHEFFER & VAN NES 2007). Climatic variations have decisive effects on the distribution of benthic organisms (SMITH et al. 2003) and can occur at different timescales, both intra-annual (seasons) and inter-annual (between years).

Chironomidae (Insecta: Diptera) occur in great abundance and high diversity in most aquatic ecosystems in all continents (EPLER 2001, FERRINGTON 2008). They play an important role in the food web of aquatic communities, establishing links between producers and consumers, as well as participating in nutrient cycles (HENRIQUES-OLIVEIRA et al. 2003). Chironomids are tolerant to various changes in the environment (ROSIN & TAKEDA 2007, RESTELLO et al. 2012), and depending on the species, they may display negative or positive responses to human impacts (FERRINGTON 2008).

Chironomid communities can be effected by the integrity of the riparian zone. The state and the extent of the riparian vegetation correlates with differences in the abundance, richnness, and composition of chironomid communities in streams (SENSOLO et al. 2012). For instance, suppression of the riparian vegetation results in decreased overall diversity and increased numbers of tolerant taxa (AL-SHAMI et al. 2010a). Different chironomids inhabit different habitats and substrates (SANSEVERINO & NESSIMIAN 2001) although they are most frequent in heterogeneous and stable environments, where they attain high diversity (ROSA et al. 2011, 2013).Temporal variations in biological communities are mainly linked to climate-related changes (e.g., temperature and rainfall). Climate affects ecological processes such as competition, predation and recruitment (GRESENS et al. 2007). In addition to climatic factors, temporal variations in the structure and composition of chironomid communities may reflect the biological characteristics of the species that compose these communities (HEINIS & DAVIDS 1993, SIQUEIRA et al. 2008) or temporal changes in physical and chemical characteristics (AL-SHAMI et al. 2010b). Natural disturbances, such as spates caused by increased rainfall in human-impacted areas may carry chemicals from adjacent areas to the streams, thus affecting chironomid communities (GRESENS et al. 2007).

In this study, the intra- and inter-annual variation of Chironomidae communities in subtropical streams was assessed over three years. We tested the hypothesis that environmental factors related to human activities may be important in structuring communities in streams. Thus, the objectives of this study were (1) to evaluate the intra and inter-annual variations in Chironomidae communities and (2) to determine whether these temporal variations are associated with environmental factors.

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MATERIAL AND METHODS

This study was conducted in the upper portion of the Uruguay River Basin in southern Brazil (27°12'59" and 28°00'47"S, 52°48'12" and 51°49'34"W, Fig. 1). The region is characterized by a subtropical climate (Koppen Cfb) with average annual rainfall of 1912.3 mm and average annual temperature of 17.6°C. The vegetation is a subtropical forest mix. It is mostly composed of species with tropical-subtropical distribution in the Upper Uruguay, and Araucaria Forest with a predominance of Araucaria (Oliveira-Filho et al. 2015). The predominant land use is intensive agricultural practice (~77% of the total area), with soybeans, corn, wheat crops, and large forested areas (Decian et al. 2009). Thus, all 10 selected streams are embedded in a complex agricultural matrix. All streams studied were small-order streams (<3rd order) and had similar limnological characteristics. The average percentage of vegetation in the riparian zone of the streams was 23% (range 11-49%).

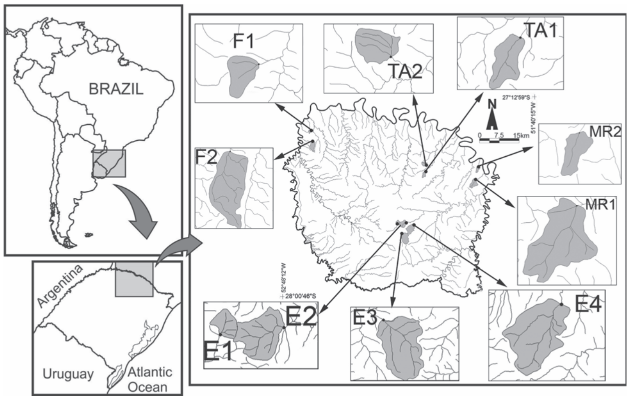


Figure 1. Geographical location of sampling sites at Alto Uruguay region, RS. F: Faxinalzinho, TA: Três Arroios, ERE: Erechim, MR: Marcelino Ramos.

We obtained the following variables from each stream: water temperature, turbidity, conductivity, total dissolved solids, dissolved oxygen and pH, with the aid of a multiparameter analyser HORIBA® U50. A Shimadzu® TOC-VCSH analyzer was used to measure total organic carbon (TOC) and total nitrogen.

Chironomidae larvae were collected in August (winter) and December (summer) of the years 2010, 2011 and 2012. At each stream, three sampling units were obtained with a Surber sampler (mesh 250 µm, area 0.09 m^2) on a rock substrate. The material was fixed in the field using 80% alcohol. In the laboratory, Chironomidae larvae were dipped in a of 10% bleach solution of potassium hydroxide for 24 hours. Individuals were then mounted on semi-permanent slides with Hoyer solution and were identified under optical microscope with a magnification of 1,000 times. Specimens were identified at the genus level using the identification keys of Trivinho-Strixino & Strixino (1995) and Trivinho-Strixino (2011).

To assess the intra- and inter-annual variations in abiotic variables Multivariate Analysis of Variance (MANOVA) was used. Variations in chironomid abundance and richness between the seasons (intra-annual) and between the years (inter-annual) were evaluated using a repeated measure Analysis of Variance (RM-ANOVA). Non-Metric Multidimensional Scaling (NMDS) (Kruskal 1964) was used to order the chironomid communities. The NMDS was performed with a biological matrix based on the presence or absence of genera in each stream using the Jaccard index. The relationship between environmental and biological data was tested by fitting vectors of environmental variables to the NMDS ordination (function 'envfit' of the vegan package). Analysis of Similarity (ANOSIM) was used to evaluate the level of segregation in community composition between years and within years,. All analyses were performed using R software (R Core Team 2013) with the 'vegan' package (Oksanen et al. 2013).

RESULTS

The studied streams have well-oxygenated (10.85 ± 2.51 mg L^{-1}), slightly acidic water (pH 6.62 ± 0.22) with electrical conductivity of 0.030 ± 0.059 mS cm^{-1} (mean of three years). The highest average turbidity was recorded in summer (11.75 ± 4.90 NTU). However, the highest average total organic carbon was recorded in winter (218.34 ± 216.16 mg L^{-1}) (Table 1). The total organic carbon was very high in the winter of 2011 (Table 1). The highest average monthly rainfall occurred in 2011 (172.20 ± 85.16 mm) followed by 2010 (122.6 ± 101.7 mm) and 2012 (36.7 ± 60.0 mm; Fig. 2). However, in 2010 and 2012there was as much rainfall in the winter and the summer. In 2011, the difference in rainfall between the winter and summer seasons was ca. 150 mm. Overall, the abiotic variables differed among the years and between the seasons ($F_{(2,56)} = 18.22$, $p = 0.001$ and $F_{(1,56)} = 25.10$, $p = 0.001$, respectively, Table 2).

We obtained a total of 7,568 chironomid larvae distributed in 49 genera. The highest abundance was recorded in 2012 (3,304 larvae, 43.7% of the total), followed by 2011 (2,430 larvae, 32.1%) and 2010 (1,834 larvae, 24.2%). In two of the three years studied (2010 and 2012, Fig. 3, Table 3) chironomids were more abundant in the winter. The greatest number of chironomid genera (43 genera) was identified in 2011, followed by 2012 (33 genera) and 2010 (25 genera). Thus, abundance varied intra-annually while richness varied intra- and inter-annually (Table 3, Fig. 4).

Among the genera identified, *Pentaneura* Philippi, 1865, *Polypedilum* Kieffer, 1912, and *Rheotanytarsus* Thienemann & Bause in Bause, 1913 were the most frequent in the samples. *Aedokritus* Roback, 1958, *Antillocladius* Saether, 1981, *Denopelopia* Roback & Rutter, 1988, *Djalmabatista* Fittkau, 1968,

Table 1. Mean and standard deviation of limnological variables quantified the drainage areas of the 10 studied streams in the region Alto Uruguay Rio Grande Sul, in the period 2010-2012.

Variables	2010		2011		2012	
	Summer	Winter	Summer	Winter	Summer	Winter
Water temperature (°C)	20.72 ± 0.87	15.05 ± 1.84	21.45 ± 3.03	14.40 ± 1.57	22.78 ± 1.98	16.31±1.79
pH	6.87 ± 0.84	6.45 ± 0.56	6.45 ± 0.59	6.89 ± 0.79	6.71 ± 0.43	6.37±0.48
Electrical Conductivity (mS cm^{-1})	0.05 ± 0.03	0.05 ± 0.02	0.08 ± 0.04	0.051 ± 0.02	1.52 ± 4.59	0.06±0.03
Turbidity (UNT)	13.37 ± 17.26	3.07 ± 8.99	6.25 ± 2.82	8.46 ± 5.05	15.65 ± 19.87	8.85±5.40
DO (mg L^{-1})	10.38 ± 1.06	8.36 ± 0.90	9.54 ± 2.57	9.49 ± 0.73	11.92 ± 3.15	15.39±1.67
TDS (mg L^{-1})	0.04 ± 0.02	0.03 ± 0.01	0.05 ± 0.03	0.03 ± 0.01	0.04 ± 0.02	0.03±0.02
Nitrogen (mg L^{-1})	15.66 ± 3.37	6.36 ± 2.80	0.44 ± 0.47	1.32 ± 0.68	1.13 ± 0.89	1.05±0.69
TOC (mg L^{-1})	17.14 ± 5.35	25.05 ± 21.80	83.90 ± 39.06	451.76 ± 100.39	61.28 ± 58.23	178.21±50.15

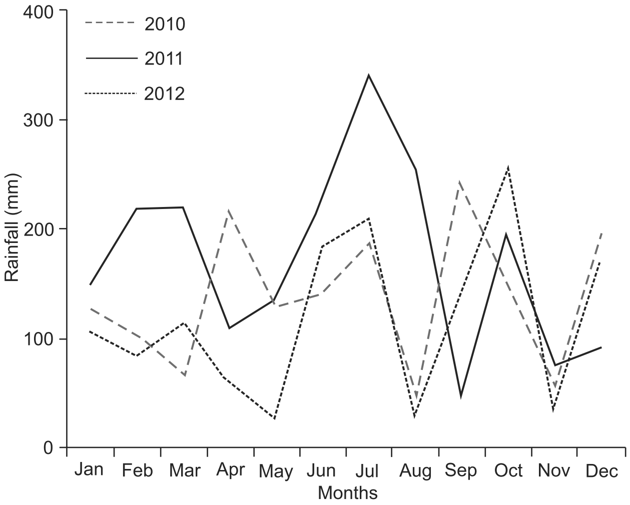
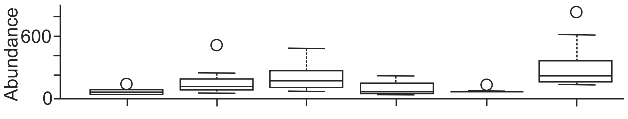


Figure 2. Monthly rainfall in the years 2010, 2011 and 2012 at Alto Uruguay region, RS. The horizontal lines indicate the annual average for the respective years (INMET).



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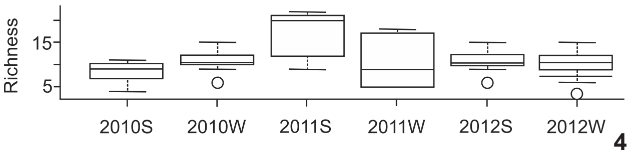

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Figures 3-4. Box-plot (median and quartiles) showing the variation of (3) and abundance (4) wealth of inter-annual chironomid larvae (2010, 2011 and 2012) and intra-annual (summer: S, winter: W) in subtropical streams.

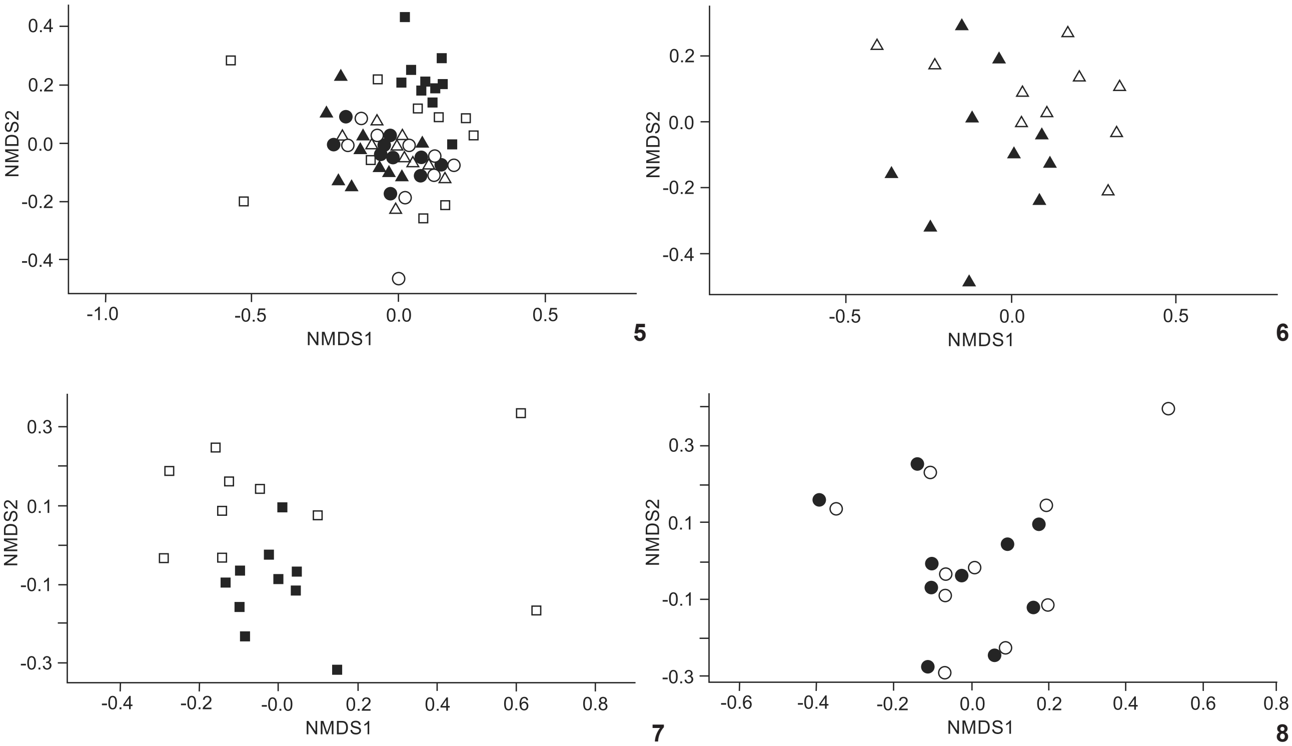
Table 2. MANOVA results for limnological intra and inter-annual among the studied streams were considered as factors the years studied (inter-annual) and the seasons (intra-annual).

	DF	SS	MS	F	p
Season	1	1.013	1.013	25.101	0.001
Year	2	1.471	0.735	18.225	0.001
Residuals	56	2.260	0.040	0.476	
Total	59	4.744			

Table 3. Repeated measures ANOVA results for the variation in the abundance (log) and richness of Chironomidae community between seasons and among years at 10 stream sites in Alto Uruguay region, Rio Grande Sul.

	DF	SS	F	p
Abundance (log)				
Year	2	1.01	0.75	0.477
Seasons	1	4.73	7.04	0.010
Year:Seasons	2	32.22	23.97	<0.001
Residuals	54	36.30		
Richness				
Year	2	235.90	8.44	<0.001
Seasons	1	43.35	3.10	0.008
Year:Seasons	2	237.90	8.51	<0.001
Residuals	54	754.50		

Manoa Fittkau, 1963, *Paracladius* Hirvenoja, 1973, *Paramerina* Fittkau & Stur, 1997, and *Pseudochironomus* Riethia Malloch, 1915 occurred only in winter, while *Endotribelos* Grodhaus, 1987, *Microchironomus* Kieffer, 1918, *Parapentaneura* Stur, Fittkau & Serrano, 2006, and *Ubatubaneura* Wiedenbrug & Trivinho-Strixino, 2009 occurred only in summer (Table 4). Community composition was similar between seasons when the three years were analysed together (ANOSIM, R = -0.01, p = 0.596,



Figures 5-8. Non-Metric Multidimensional Scaling Analysis (NMDS) of the temporal distribution of chironomid community in subtropical streams. (5) inter-annual distribution (2010, 2011, 2012), (6) intra-annual distribution for 2010, (7) intra-annual distribution for 2011, (8) intra-annual distribution for 2012. Open symbols: winner, closed symbols: summer.

Figs. 5-8). However, the generi composition showed segregation between summer and winter in 2011 (ANOSIM, R = 0.32, p = 0.001) and 2010 (ANOSIM, R = 0.12, p = 0.034), but not in 2012 (ANOSIM, R = -0.09, p = 0.976) (Figs. 5-8).

The environmental variables that were correlated with the scores of the first two NMDS dimensions were those that variated seasonally (temperature), human activities (total organic carbon and nitrogen) and rainfall (Table 5). When data from all years were pooled, the community composition correlated with C:N ratio (r = 0.09, p = 0.05) and rainfall (r = 0.11, p = 0.04). Moreover, in 2010, the NMDS scores showed significant correlation with temperature (r = 0.31, p = 0.04) and nitrogen (r = 0.31, p = 0.03). In 2011 there was a relationship between water temperature (r = 0.47, p = 0.004), nitrogen (r = 0.42, p = 0.009), TOC (r = 0.35, p = 0.02) and rainfall (r = 0.30, p = 0.03). In 2012 there was no correlation with any environmental variable (Table 5).

DISCUSSION

Chironomidae abundance and generic richness varied either intra-annually (between seasons) and inter-annually (between years). In our study, the abundance of individuals and number of chironomid genera were associated with limnological characteristics. The effect of these characteristics on the chironomid community may have occurred in association with a significant rainfall event in 2011. Increased precipitation causes changes in aquatic ecosystems, such as changes in the physical and chemical characteristics of the water, as well as changes in the distribution of communities (Smith et al. 2003). When rainfall increases, chemical compounds present in the soil are dragged into the streams and cause changes in the abundance, richness, and composition of chironomid communities (Gresens et al. 2007). The effect of runoff in this study was especially observed in TOC concentrations in the winter of 2011.

In 2010 and 2012 rainfall was normal with respect to historical records. Streams and their chironomid communities were stable as a result of this. Rainfall increases the amount of water and unstable and homogeneous substrates into the streams (Rosa et al. 2013, Salles & Ferreira-Junior 2014). Therefore, the increased rainfall (2011) during the study period was an important contributor to the observed variations in inter-annual richness. Fluctuations in water regimen allow great habitat diversification, i.e. more tolerant species can occupy distinct regions of the drainage area, in addition to changing the limnological characteristics of streams (Shuvart et al. 2005, Aburaya & Callil 2007, Roque et al. 2007). Silva et al. (2014) also observed that Chironomidae diversity was higher during the years when the abiotic charac-

Table 4. Chironomidae identified intra-annual (2010-2012) and inter-annual (summer and winter) in subtropical streams.

	2010		2011		2012	
	S	W	S	W	S	W
Chironominae						
Aedokritus Roback, 1958				*		
Caladomyia Säwedal, 1981	*	*	*		*	*
Chironomus Meigen, 1803			*	*		
Dicrotendipes Kieffer, Epler, 1988			*	*		
Endotribelos Grodhaus, 1987			*			
Goeldichironomus Fittkau, 1965	*		*			
Manoa Fittkau, 1963				*		
Microchironomus Kieffer, 1918			*			
Parachironomus Lenz, 1921	*		*			
Paratanytarsus Thienemman & Bause, 1951			*	*		
Paratendipes Kieffer, 1911	*	*	*	*	*	*
Phaenopsectra Kieffer, 1921	*		*	*		
Polypedilum Kieffer, 1912	*	*	*	*	*	*
Pseudochironomus, Riethia Malloch, 1915		*	*	*	*	*
Rheotanytarsus Thienemann & Bause, 1913	*	*	*	*	*	*
Saetheria Saether, 1983			*			
Stenochironomus Kieffer, 1919			*			
Tanytarsus Van der Wulp, 1874	*	*	*	*	*	*
Xestochironomus Sublette & Wirth, 1972			*			
Zavreliella Kieffer, 1920			*	*		
Orthocladiinae						
Antillocladius Saether, 1981				*		
Cardiocladius Kieffer, 1912			*	*		
Corynoneura Winnertz, 1846	*	*	*	*	*	*
Cricotopus Van der Wulp, 1874	*	*	*		*	*
Cricotopus, Orthocladius Lopescladius Oliveira, 1967	*	*	*	*	*	*
Gymnometriocnemus Goetghebuer, 1932	*	*	*			
Lopescladius Oliveira, 1967	*	*	*		*	*
Metriocnemus Kieffer 1921			*	*		
Nanocladius Kieffer, 1912	*	*	*	*	*	*
Onconeura Andersen & Saether, 2005	*	*	*	*	*	*
Orthocladiinae A Kieffer, 1911			*	*		
Orthocladiinae B Kieffer, 1911			*	*		
Paracladius Hirvenoja, 1973			*			
Parakiefferiella Thienneman 1926		*	*	*	*	*
Parametriocnemus Goetghebuer, 1932	*	*	*	*	*	*
Paraphaenocladius Thienemann, 1924			*	*		
Rheocricotopus Thienemann & Harnisch, 2004	*	*	*	*	*	*
Thienemannia Kieffer, 1909			*	*		
Thienemanniella Kieffer, 1911		*	*	*	*	*
Ubatubaneura Wiedenbrug & Trivinho-Strixino, 2009			*			
Tanypodinae						
Denopelopia Roback e Rutter (1988)				*		
Djalmabatista Fittkau (1968)			*			
Hudsonimyia Roback, 1979			*	*		
Labrudinia Fittkau, 1962			*			
Larsia Roback & Coffman (1989)			*	*		
Nilotanypus Kieffer, 1923	*	*	*	*	*	*
Paramerina Stur and Fittkau, 1997				*		
Parapentaneura Stur, Fittkau & Serrano, 2006			*			
Pentaneura Philippi, 1865	*	*	*	*	*	*

Table 5. Analysis of the structure between the abiotic data and the biological matrix (NMDS), inter- and intra-annual variation tested from the non-parametric multivariate analysis in subtropical streams.

	NMDS1	NMDS2	R2	p
2010 to 2012				
Water temperature	0.331	0.943	0.078	0.12
pH	-0.967	-0.251	0.094	0.058
Electrical Conductivity	0.559	-0.828	0.011	0.669
Turbidity	-0.812	-0.583	0.016	0.575
DO	-0.168	-0.985	0.088	0.088
TDS	-0.125	0.992	0.042	0.285
Nitrogen	-0.999	0.043	0.041	0.287
TOC	-0.968	-0.247	0.004	0.907
C:N ratio	-0.288	0.957	0.099	0.050
Water velocity	0.891	-0.452	0.016	0.551
Rainfall	-0.847	-0.531	0.110	0.041
2010				
Water temperature	-0.834	-0.551	0.310	0.040
pH	-0.898	0.439	0.118	0.364
Electrical Conductivity	-0.990	-0.134	0.092	0.457
Turbidity	-0.163	-0.986	0.071	0.535
DO	-0.426	-0.904	0.285	0.064
TDS	-0.880	-0.473	0.111	0.378
Nitrogen	-0.711	-0.702	0.316	0.039
TOC	-0.609	0.792	0.284	0.057
C:N ratio	-0.408	0.912	0.260	0.090
Water velocity	0.749	-0.661	0.062	0.588
Rainfall	-0.695	-0.718	0.051	0.626
2011				
Water temperature	-0.039	-0.999	0.474	0.004
pH	0.590	0.806	0.091	0.441
Electrical Conductivity	0.335	-0.941	0.107	0.378
Turbidity	-0.523	0.851	0.238	0.105
DO	-0.271	0.962	0.065	0.527
TDS	0.342	-0.939	0.110	0.371
Nitrogen	-0.328	0.944	0.426	0.009
TOC	0.113	0.993	0.352	0.021
C:N ratio	0.980	0.194	0.196	0.156
Water velocity	-0.579	0.814	0.065	0.462
Rainfall	0.134	0.990	0.309	0.036
2012				
Water temperature	0.986	-0.165	0.007	0.938
pH	0.753	0.658	0.017	0.854
Electrical Conductivity	0.291	-0.956	0.097	0.338
Turbidity	-0.785	0.618	0.226	0.114
DO	0.947	0.320	0.006	0.949
TDS	0.917	0.397	0.023	0.832
Nitrogen	0.948	0.315	0.012	0.903
TOC	0.142	0.989	0.075	0.543
C:N ratio	-0.671	0.741	0.249	0.104
Water velocity	0.332	0.943	0.039	0.726
Rainfall	0.705	0.708	0.019	0.855

teristics of their studied lake changed. Rainfall is one abiotic variable that can create favorable conditions for certain species, not only as a function of the new habitat conditions (Bispo et al. 2006). Water temperature affects the metabolism of organisms and the availability of food, causing changes in community composition (Hahn & Figi 2007, Gray & Elliott 2009). The highest concentrations of carbon and nitrogen were found in areas with intense human activity (e.g. agricultural practices) (Neill et al. 2001, Silva et al. 2007). Currently, many streams display similar signs of anthropogenic change (mainly as the result of agricultural practices), a phenomenon known as eutrophication of aquatic ecosystems (Galloway et al. 2003, Silveira et al. 2006). The drainage areas of the streams studied were populated with crops and exposed soil (in preparation for cultivation). In these areas, the use of pesticides and fertilizers is high, causing soil contamination particularly when rainfall is intense. These pesticides, plus organic matter and nutrients, are carried into the streams by the rain water.

Organic matter is primarily composed of carbon, but it can be associated with other chemical compounds, such as metals (Ali et al. 2002, Al-Shami et al. 2010a, Sensolo et al. 2012). On the other hand, nitrogen is among the most limiting nutrients to primary productivity and the availability of this nutrient affects the abundance of some aquatic organisms (Galloway et al. 2003). *Cricotopus* species feed on algae, which in turn are dependent on certain concentrations of dissolved nutrients (Sensolo et al. 2012). *Polypedilum* and *Rheotanytarsus* were the most common organisms in all samples. Studies report that these genera are easily sampled in streams and are reported as cosmopolitan/tolerant (Amorim et al. 2004, Marchese et al. 2005, Aburaya & Callil 2007). Furthermore, the high density of individuals of *Rheotanytarsus* is due to their eating habits: they are filter-feeding organisms, consuming exclusively organic matter present in the water (Coffman & Ferrington 1996). *Polypedilum* species stand out for being tolerant to a wide range of environmental conditions, as they may occur both in sites impacted with organic compounds and in non-impacted sites (Heinis & Davids 1993, Rosin & Takeda 2007).

In conclusion, in small temporal scales, local environmental factors have great relative influence on community composition. On the other hand, in larger timescales, climatic factors generate variation (Silva et al. 2014). In this study, we observed that in those three years, with semi-annual collecting, intra-annual variations are most evident in the chironomid communities. In this study, the temporal variation of the community was dependent on climatic variations (rainfall) as well as the changes in the TOC and TN caused by intensive agricultural land use.

ACKNOWLEDGMENTS

DN received financial support from the Program PROSUP/CAPES. RMR receives financial support from CNPq (Proc. 477274/2011-0 and proc. 475251/2009-1). LUH receives financial support from CNPq (Edital Universal, process 471572/2012-8) and FAPERGS (process 12/1354-0). The authors thank Rodrigo Fornel for their help editing images, and Adriano Melo and two anonymous reviewers for their suggestions and criticisms.

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Submitted: 30 November 2014
Received in revised form: 17 April 2015
Accepted: 2 May 2015
Editorial responsibility: Adriano S. Melo