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Longitudinal variation of attributes from flagellate protozoan community in tropical streams

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ABSTRACT. This study verified the existence of longitudinal patterns in species composition, richness, density and biomass of flagellate protozoan in tropical streams and investigated whether the possible zonation patterns are different between two periods of the year. For this, samplings were carried out in three regions from 10 streams, during the summer and winter. The flagellate community may be considered species-rich, because it was represented by 106 taxa, belonging to 8 orders and 1 residual group. The values of density and biomass are greater than those commonly found in other lotic environments, with mean values close to 2.3×10^4 cels. mL^{-1} and 150.8 $\mu gC\ L^{-1}$. We did not observe any conspicuous and significant longitudinal pattern of the attributes from flagellates community. Only temporal variations of these attributes were verified. The Pearson Correlation evidenced that this temporal patterns was mainly driven by the nutrients availability, temperature and dissolved oxygen, since, the higher values of species richness, density and biomass were recorded during the winter, when the higher concentrations of nutrients and dissolved oxygen and lower temperatures were registered. In summary, the absence of patterns may be ascribed to the unidirectional and continuous flow from lotic environments.

Keywords: protozooplankton, community structure, zonation, lotic environments.

RESUMO. Variação longitudinal dos atributos da comunidade de protozoários flagelados de riachos tropicais. O presente estudo objetivou verificar a existência de padrões longitudinais de composição, riqueza de espécies, densidade e biomassa da comunidade de protozoários flagelados de riachos tropicais e, ainda, investigar se os possíveis padrões de zonação são diferentes entre dois períodos do ano. Foram realizadas coletas em três regiões ao longo de dez riachos, durante os períodos de verão e inverno. A comunidade de protozoários flagelados pode ser considerada bastante rica, sendo representada por 106 táxons pertencentes a oito ordens e um grupo residual. Os valores de densidade e biomassa registrados encontram-se acima dos valores comumente encontrados em outros ambientes lóticos, com valores médios próximos de $2,3 \times 10^4$ cels. mL^{-1} e 150,8 $\mu gC\ L^{-1}$. Não foi verificado nenhum padrão longitudinal conspícuo e significativo dos atributos dessa comunidade. Foram verificadas apenas variações temporais destes atributos. As correlações de Pearson demonstraram que esse padrão temporal foi governado principalmente pela disponibilidade de nutrientes, temperatura e oxigênio dissolvido, visto que os maiores valores de riqueza, densidade e biomassa foram registrados no inverno, e também foram verificadas as maiores concentrações de nutrientes, de oxigênio dissolvido e as menores temperaturas. Em suma, sugere-se que essa ausência de padrões longitudinais pode ser atribuída ao fluxo unidirecional e contínuo dos ambientes lóticos.

Palavras-chave: protozooplâncton, estrutura de comunidade, zonação, ambientes lóticos.

Introduction

Rivers and streams have been considered as open, unidirectional, structurally unstable ecosystems, dynamic in space and in time and with constant interactions with the terrestrial system (STANFORD; WARD, 1993; GILLER; MALMQVIST, 1998). Due to these traits, these ecosystems support a unique and highly specialized biota (GILLER; MALMQVIST, 1998). Several authors state that these ecosystems must be studied in three spatial dimensions: horizontal, vertical and lateral (GILLER; MALMQVIST, 1998; PETTS, 2000). Meanwhile, the horizontal dimension is one of the main studied (WARD; TOCKNER, 2001; THORP et al., 2006), probably because among the most important theories developed in rivers, the river continuum concept (VANNOTE et al., 1980) was one of the first to be postulated.

According to this concept, the rivers from headwaters to mouth, present a continuum gradient of environmental conditions that may predict several responses from populations, resulting in a continuum biotic adjustment and also in effective rates of removal, displacement, use and storage of organic matter along the course of one river (VANNOTE et al., 1980).

Although this concept has been developed for not disturbed rivers from temperate regions, and of several orders, it furnishes important theoretical basis for the study of patterns of longitudinal zonation in biological communities from tropical lotic environments. However, these patterns had not been frequently investigated in these environments and most studies were accomplished with macroscopic organisms, especially, with benthic macroinvertebrates. Studies that had investigated specifically the patterns of longitudinal distribution of flagellate protozoans in streams were not subject of previous studies.

Thus is of great relevance to know these patterns, since these organisms play important role in nutrients re-mineralization and in organic matter cycling (AZAM et al., 1983; WEISSE, 1991), as they are consumed by microzooplankton and benthic predators (JÜRGENS et al., 1994; WEISSE, 1990; 1991) they transfer to upper trophic levels the energy obtained in the consumption of bacteria, pico and nanophytoplankton and even the dissolved organic carbon (SHERR; SHERR, 1992; XU et al., 2005).

In this way, the present study verified the existence of longitudinal patterns in species composition, richness, density and biomass of flagellate protozoan from tropical streams and investigated whether the possible zonation patterns are different between summer and winter. For this, we predict that: i) the patterns of species composition and richness suffer changes along the course of the streams due to the longitudinal changes in physical and chemical characteristics from the environment; ii) density and biomass from autotrophic fraction are higher in the middle course of the streams, whereas, the density and biomass from heterotrophic fraction are higher at headwater and mouth regions. This expected pattern may be explained by the fact that in headwater and mouth regions, the light penetrations is lower due to the shading and turbidity increase, respectively. Furthermore, the headwaters receive higher loads of allochtonous organic matter which may favor the heterotrophic fraction.

Material and methods

Study area

The Pirapó river catchment area (22°30'S and 23°30'S; 51°15'W and 52°15'W) is located in the physiographic region of Third Plateau of Paraná, Paraná State, Brazil. This watershed has a drainage area of about 5,076 km^2 and is under strong influence of Maringá County that is relatively industrialized and urbanized, and is considered as the most important urban center of the region. The streams studied (Água do Pirapó, Água Queçaba, Água da Roseira, Córrego do Remo, Guaiapó, Mandacarú, Miosótis, Nazaré, Romeira and Zaúna) are small orders (*Sensu* STRAHLER, 1957) and are situated near Maringá County, consequently, they receive direct influence from urban area and from agricultural activities developed in the region (Figure 1).

Samplings and laboratory analysis

Samplings were performed during two periods of the year, one in July, 2007, during the winter, and the other in February, 2008, during summer. The samplings were carried out under water surface, in triplicate, in three regions (headwater, middle course and mouth) from each stream, totaling 360 samples.

Electric conductivity, dissolved oxygen, pH, depth, water temperature and current velocity were measured in field, using portable equipments. The concentrations of nitrate and phosphate were determined in laboratory using the methods proposed by Zagatto et al. (1981) and Golterman et al. (1978), respectively.

Nanoflagellate samples were obtained through passage of a jar under water surface of streams. Afterwards, a portion (100 mL) was preserved using the solution compounded by formaldehyde, alkaline lugol and tiosulfate and the other portion (2 L) was analyzed in vivo, under optical microscope, for species identification. The slides were analyzed until no new species arose during this analysis. The specimens were identified, whenever possible, at species level, based on morphological traits using specialized literature. The results were presented according to the classification system developed by Lee et al. (2000).

To determine density, biomass and body size of nanoflagellate, semi-permanent slides were set up through the filtering preserved samples (25 mL) in black membrane Nucleopore/Watchman (0.8 μm), previously stained with DAPI (4,6'-diamidino-2-phenylindole, at 0.1%). Countings were performed in epifluorescence microscope at 400x magnitude, where 25 fields or 300 cells (> 7 e < 30 μm) were estimated and afterwards at 1000x magnitude, where 100 fields or 300 cells (< 7 μm) were counted.

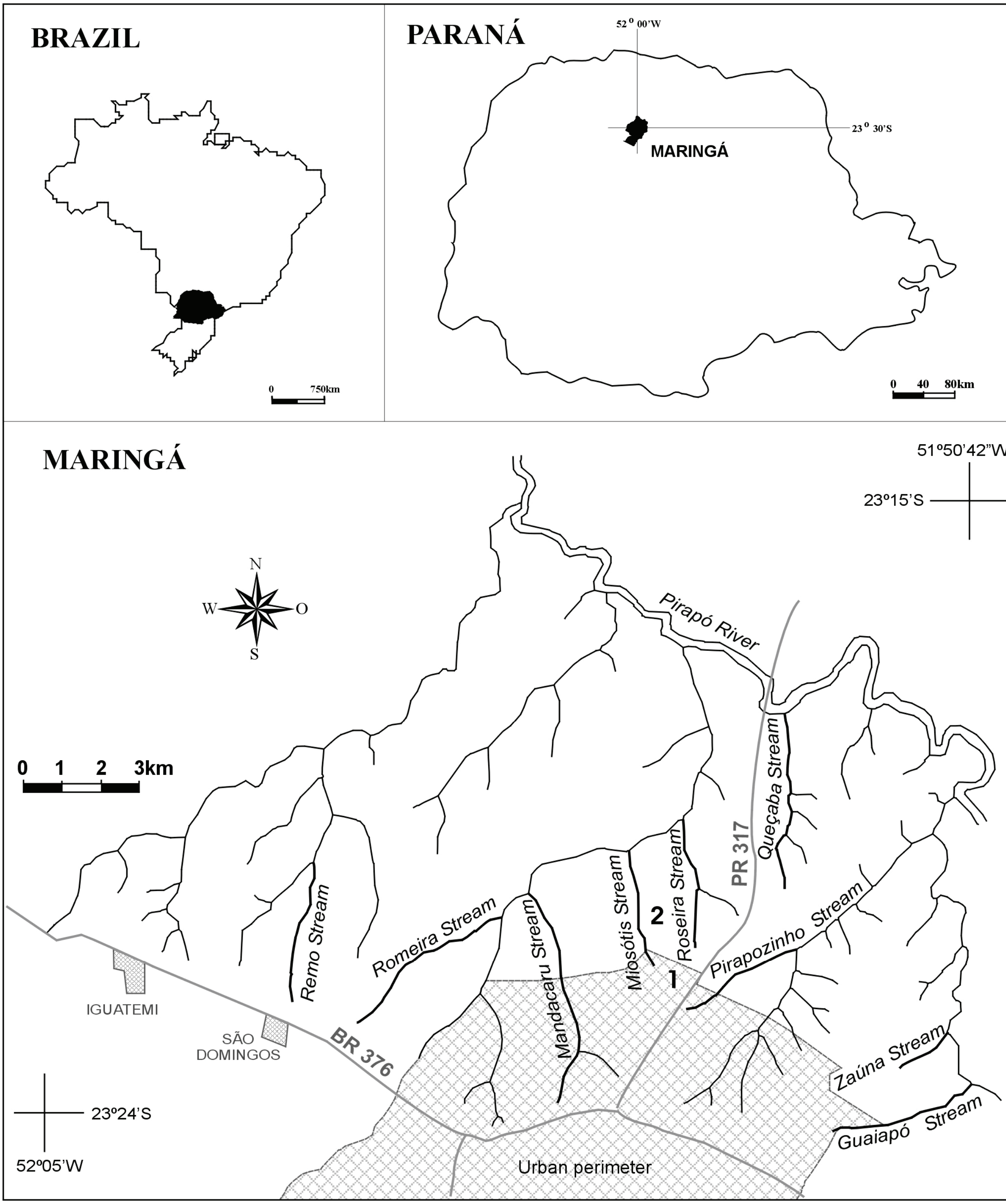

Figure 1. Location of study area (1: urban region; 2: rural region).

Concomitantly to the countings, the individuals were measured using micrometer ocular to determine the cell biovolume, through measurements of cell dimensions and approximate geometrical shapes (WETZEL; LIKENS, 1991). The carbon content was determined according to the conversion factor proposed by Fenchel (1982), where 1 μm^3 = 167 fg C. The differentiation between the autotrophic and heterotrophic nanoflagellate was made under ultraviolet light, where the heterotrophic organisms are visualized with green color and the autotrophic ones with red or orange color.

Data analysis

The abiotic variables were summarized through the principal component analysis (PCA) aiming to characterize the streams and detect a possible environmental gradient important for the structure of nanoflagellate community. The axes were retained for interpretation according to the Kaiser Guttman criteria (Eigenvalues > 1.0) (JACKSON, 1993).

The spatial and temporal patterns of species composition were evaluated through the detrended correspondence analysis (DCA), which was performed with data of presence-absence of flagellate protozoans with frequency of occurrence superior to 5%. Afterwards, we applied a bi-factorial analysis of variance to the scores of each axis, to test differences in species composition among the regions of the streams and between the periods.

In order to verify the existence of significant differences in the attributes from nanoflagellate community among the regions of each stream and between the periods, bi-factorial analysis of variance were also employed.

Pearson correlation analyses were performed to infer about the relationships between density, biomass, species composition and richness and abiotic variables (represented by the PCA scores).

Before the analysis, all data (except for pH and presence-absence data) were log transformed (log x+1) aiming to minimize the variance. The assumptions were also tested and reached. The analysis of variance and the Pearson correlations were made using Statistica 7.0 software (STATSOFT, 1996). PCA was accomplished using Past software (HAMMER et al., 2001), and DCA, using PC-ORD version 4.0 (McCUNE; MEFFORD, 1999).

Results

Environmental variables

Mean values of abiotic variables, obtained in three regions of studied streams, are summarized in table 1. In general, the studied streams are shallow, with high current flow, slightly acid pH, relatively low concentrations of dissolved oxygen, high electric conductivity, and high nutrients concentrations, especially nitrate (Table 1). In relation to spatial and temporal variation of these variables, we did not detect great oscillation in the mean values among the regions; however, the mean values between the periods presented more evident variations (Table 1).

The abiotic variables were summarized through principal component analysis and the four first axes were retained for interpretation and explained 70% of total data variability (Table 2; Figures 2a and b). However, great part of data variability (41.5%) was explained by axes 1 and 2, which clearly evidenced the distinction of environments (Figure 2a, Table 2). The axis 1 was negatively influenced by electric conductivity, water temperature and depth, and positively by current velocity and dissolved oxygen (Figure 2a, Table 2). The axis 2, in turn, was negatively influenced by phosphate, dissolved oxygen and pH (Figure 2a, Table 2). In general, the axis 1 distinguished the spatial variation in abiotic variables and the axis 2, the temporal variation. The variables that most influenced the formation of the rest of the axes retained for interpretation are listed in Table 2.

Species composition

The flagellate community was represented by 106 taxa, belonging to 8 Orders: Chromulinales (6 taxa), Cryptomonadida (5 taxa), Euglenida (71 taxa), Gymnodiniales (5 taxa), Kinetoplastea (8 taxa), Peridiniales (2 taxa), Synurales (2 taxa), Volvocida (6 taxa) and 1 residual taxa. The Euglenida was the most species-rich Order in all regions of the streams (Figure 3), due to contribution of *Trachelomonas* (16 taxa), *Euglena* (11 taxa) and *Phacus* (11 taxa).

Table 1. Mean values and standard deviation from physical, chemical and biological parameters in three regions from studied streams, during the summer and winter.

Parameters	Winter						Summer					
	Headwater		Middle course		Mouth		Headwater		Middle course		Mouth	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Electric conductivity (μS cm^{-1})	147.49	64.25	159.15	53.70	143.11	38.63	164.26	56.53	159.13	13.16	147.28	8.14
Phosphate (mg L^{-1})	0.14	0.16	0.08	0.10	0.14	0.18	0.07	0.05	0.09	0.01	0.06	0.01
Nitrate (mg L^{-1})	8.96	2.56	9.38	2.02	9.80	3.25	3.30	2.29	4.18	0.69	2.93	0.35
Dissolved oxygen (mg L^{-1})	8.05	0.50	8.51	0.58	8.76	0.46	7.17	0.52	7.07	0.16	7.49	0.08
pH	7.83	1.93	7.60	1.03	7.35	0.57	6.92	0.23	6.87	0.04	6.90	0.05
Depth (m)	0.21	0.09	0.22	0.10	0.25	0.13	0.23	0.11	0.19	0.02	0.20	0.02
Water temperature (°C)	18.51	2.38	17.63	1.60	16.68	1.33	18.43	1.42	19.15	0.33	18.29	0.25
Current velocity (m.s^{-1})	0.26	0.15	0.32	0.14	0.29	0.14	0.27	0.13	0.25	0.03	0.32	0.03

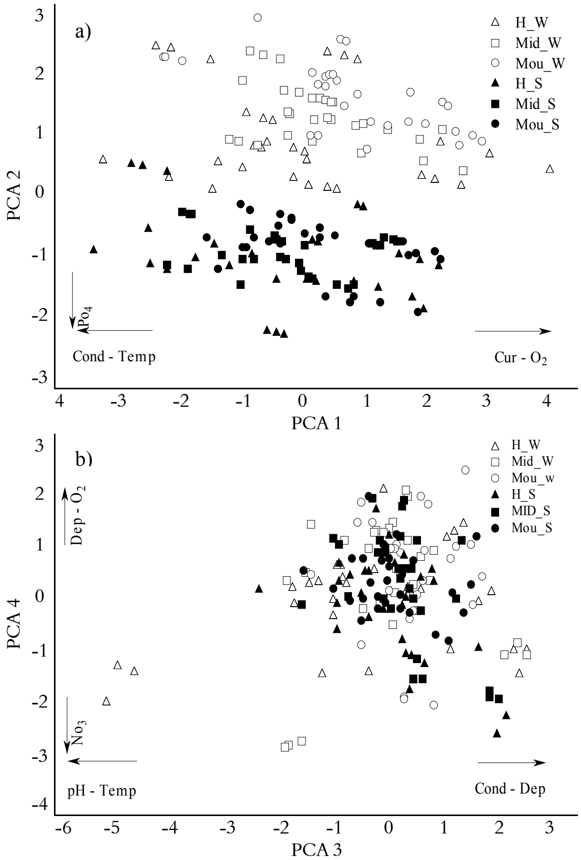

Figure 2. a) Dispersion of scores along axes 1 and 2 from PCA; b) dispersion of scores along axes 3 and 4 from PCA carried out with the limnological variables in three regions along the course of studied streams, during the summer and winter (Where: Cond = Electric conductivity; Cur = Current velocity; NO_3 = Nitrate; O_2 = Dissolved oxygen; Po_4 = Phosphate; Dep = Depth and Temp = Water temperature; and H_S: headwater in summer; Mid_S: middle course in summer; Mou_S: mouth in summer; H_W: headwater in winter; Mid_W: middle course in winter; Mou_W: mouth in winter).

Table 2. Pearson correlation between the variables that most contributed for the formation of the axes, eigenvalues and percentage of explanation of principal component analysis (PCA), applied to limnological variables from urban and rural streams, during the summer and winter.

Abiotic variables	Axis 1	Axis 2	Axis 3	Axis 4
Electric conductivity (μS cm^{-1})	-0.62	-0.10	0.31	-0.36
Phosphate (mg L^{-1})	-0.45	-0.77	-0.07	-0.16
pH	-0.29	-0.43	-0.68	0.07
Dissolved oxygen (mg L^{-1})	0.42	-0.76	0.09	0.34
Nitrate (mg L^{-1})	0.33	-0.17	-0.08	-0.71
Water temperature (ºC)	-0.53	0.37	-0.59	-0.0008
Depth (m)	-0.49	-0.001	0.22	0.54
Current velocity (m s^{-1})	0.56	0.07	-0.45	0.15
Explanation %	22.4	19.2	14.8	13.7
Eigenvalues	1.79	1.53	1.18	1.09

The detrended correspondence analysis did not indicate a remarkable distinction among the regions neither between the periods (Figure 4), meantime, the analysis of variance applied to the scores from DCA1 and DCA2 evidenced significant differences between summer and winter for the DCA2 (Eigenvalue = 0.52; F = 9.951; p = 0.001).

Species richness

During winter, the values of species richness ranged from 1 to 22 taxa, with higher values recorded at headwater (Figure 5). On the other hand, in summer, the values of species richness varied between 1 and 11 taxa, with higher values in the middle course (Figure 4). Nevertheless, the analysis of variance pointed out that species richness is not different among regions (d.f. = 2; F = 0.24; p = 0.785), only between periods (d.f. = 1; F = 18.73; p < 0.00001).

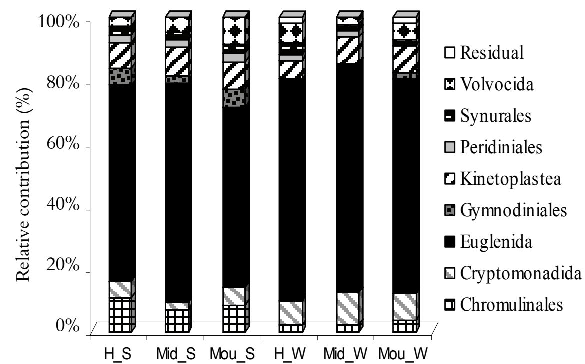

Figure 3. Relative contribution (%) of Orders to the species composition in three regions of the studied streams during the summer and winter. Where: H_S: headwater in summer; Mid_S: middle course in summer; Mou_S: mouth in summer; H_W: headwater in winter; Mid_W: middle course in winter; Mou_W: mouth in winter.

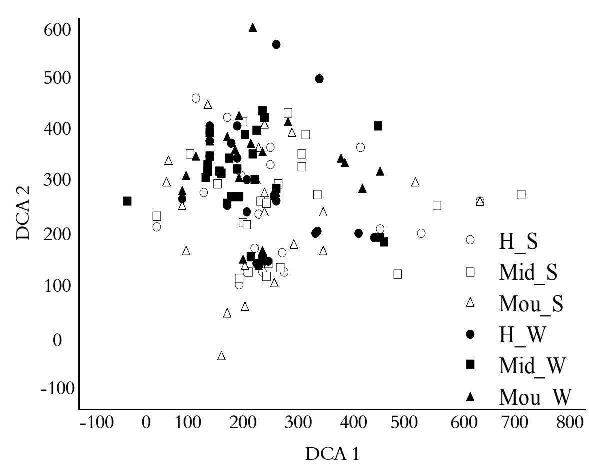

Figure 4. Dispersion of scores along two first DCA axes, based on presence-absence data from flagellate protozoan with frequency of occurrence superior to 5% in three regions along the course of studied streams, during the summer and winter. Where: H_S: headwater in summer; Mid_S: middle course in summer; Mou_S: mouth in summer; H_W: headwater in winter; Mid_W: middle course in winter; Mou_W: mouth in winter.

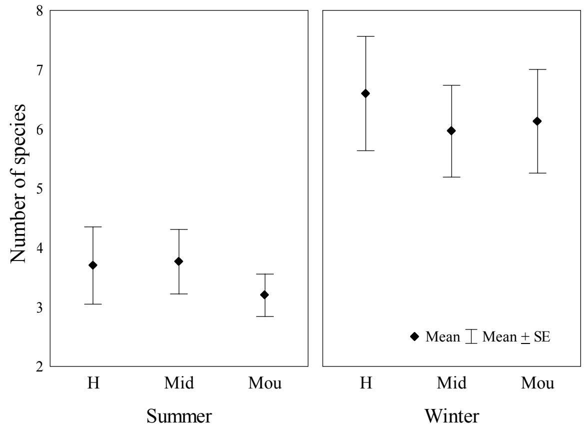

Figure 5. Species richness of flagellate protozoans in three regions along the course of studied streams, during the summer and winter. Where: H: headwater; Mid: middle course; Mou: mouth.

Density

In general, the values of total density, as well as the density values of autotrophic and heterotrophic fractions were higher at the middle course of the streams during both periods (Figures 6a and b), with mean values around 2.3×10^4 cels. mL^{-1}, 3.0×10^3 cels. mL^{-1} and 2.0×10^4 cels. mL^{-1}, respectively. Meanwhile, the analysis of variance indicated that the total density was significantly different only between periods (d.f. = 1; F = 4.729; p = 0.031). Regarding the fractions density, only the density of autotrophic fraction presented significant differences among regions (d.f. = 2; F = 3.143; p = 0.04) and between periods (d.f. = 1; F = 9.804; p = 0.002), since the density of heterotrophic fraction was not different among regions (d.f. = 2; F = 2.14; p = 0.1205) and neither between periods (d.f. = 1; F = 2.01; p = 0.1582).

Biomass

Higher values of total biomass and biomass of autotrophic and heterotrophic fractions, in general, were registered at the middle course of the streams during both studied periods (Figures 7a and b), with mean values close to 150.8 μgC L^{-1}, 55.4 μgC L^{-1} and 106.7 μgC L^{-1}, respectively. Nevertheless, this trend was not corroborated by the analyses of variance, since the total biomass (d.f. = 1; F = 23.72; p = 0.000002), autotrophic biomass (d.f. = 1; F = 25.25; p = 0.000001) and heterotrophic biomass (d.f. = 1; F = 9.84; p = 0.002) presented significant differences only between studied periods.

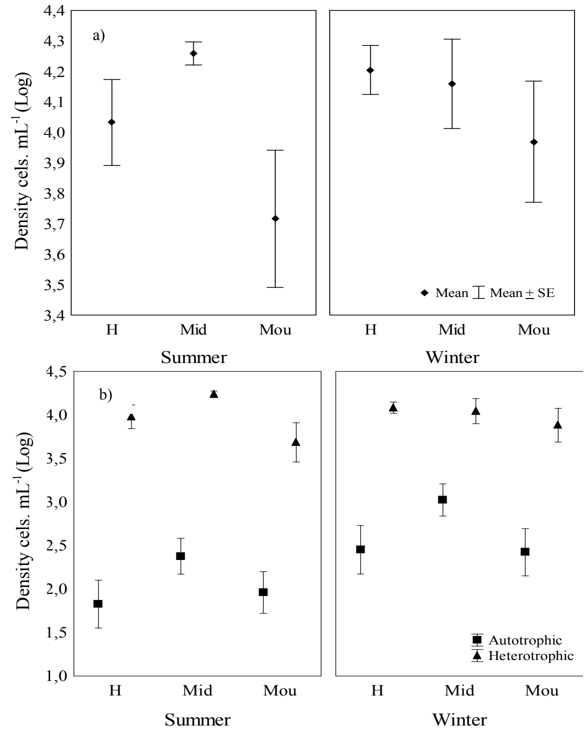

Figure 6. a) Total density of community and b) density from autotrophic and heterotrophic nanoflagellates, in three regions along the course of studied streams, during the summer and winter. Where: H_S: headwater in summer; Mid_S: middle course in summer; Mou_S: mouth in summer; H_W: headwater in winter; Mid_W: middle course in winter; Mou_W: mouth in winter.

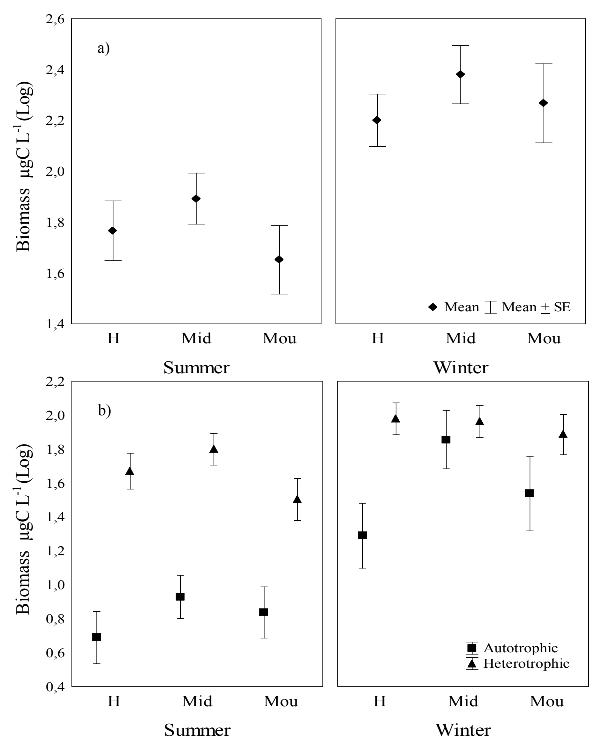

Figure 7. a) Total biomass of community and b) biomass of autotrophic and heterotrophic nanoflagellates, in three regions along the course of studied streams, during the summer and winter (mean and standard error).

Influence of abiotic variables on the flagellate distribution patterns

Pearson correlations accomplished between the attributes of flagellate community and the scores of principal component analysis were presented in Table 4. In general, this analysis evidenced that the water temperature, electric conductivity and nutrients concentrations were the most important abiotic variables to determine the spatial and temporal variations of attributes from flagellate protozoan community.

Table 4. Pearson correlations performed between the attributes from flagellate protozoan community and the scores of principal component analysis undertaken to summarize the abiotic variables from studied streams, during the summer and winter. * Significant values ($p < 0.05$).

Variables	PCA1		PCA 2		PCA 3		PCA 4	
	r	p	r	p	r	p	r	p
Density	-0.20*	0.017	0.05	0.544	-0.06	0.397	0.08	0.259
Biomass	0.12	0.121	0.22*	0.003	-0.11	0.159	-0.08	0.278
Species richness	0.06	0.461	-0.28*	0.000	-0.08	0.275	0.17*	0.023
Composition	-0.03	0.684	0.19*	0.011	-0.31*	0.000	-0.20*	0.006

Discussion

The flagellate protozoan community from the studied streams presented a much rich species composition, with the occurrence of 8 Orders and 1 residual group. The Euglenida Order was responsible for this great richness, because it contributed with 65% from the total identified species. The great success of these organisms in the streams may be associated to the human activities developed in the regions, since the euglenideans are considered as good indicators of pollution, because they prefer environments rich in organic matter, ammonia and with high biochemical oxygen demand (REYNOLDS et al., 2002).

The patterns of species composition and richness found in the present study are much different from the patters recorded by other authors in several rivers and lakes from temperate region. In general, within these environments, the number of species registered is quite low and the Chrysomonadida Order is the most representative in these studies. Furthermore, *Spumella* spp. is the most common species found by these authors (AUER; ARNDT, 2001; COMTE et al., 2006; LAVRENTYEV et al., 2004; TIKHONENKOV; MAZEI, 2006; 2008; WEITERE; ARNDT, 2003).

Moreover, the results obtained in the present study evidenced that species composition and richness did not present conspicuous and significant differences among the regions of the streams, only between the periods, thus, the first hypothesis was refuted, because these attributes presented only seasonal alterations, that probably are related both to the seasonal variations in abiotic variables and to the interactions with the other components from microbial chains.

According to Pearson correlations, the temporal variation in species richness was directly associated to the temporal variation of nutrients concentrations, since the highest values of species richness were observed concomitantly to the higher concentrations of nutrients, especially nitrate. This positive effect of nutrients on the patterns of species richness from flagellate protozoans had never been registered.

In the same way, the species composition also presented only seasonal alterations, among these; we should stress the increase in the contribution of Euglenida Order and the decrease in the contribution of Chromulinales, Gymnodyniales and Peridiniales Orders in all regions during the winter. Based on Pearson correlations, the probable causes of this higher contribution of Euglenida Order during winter are the higher values of phosphate, nitrate and dissolved oxygen, which may favor these organisms in this period.

In contrast, during summer a series of unfavorable characteristics of the medium (mainly caused by rainfall regime), as more acidic pH, low availability of oxygen, and higher temperatures may have unsettled the protozoan community structure, since in this period there was reduction in the dominance of order Euglenida, which allowed a higher occurrence of other orders in this period. These results are strengthened by the fact of low availability of oxygen and high temperatures are considered the main limiting factors for the flagellate community (ARNDT et al., 2000).

Regarding the abundance patterns from this community, the results allow inferring that the observed values of density and biomass are above to those commonly registered in other lotic environments. In these environments, the values of density and biomass ranged from 0.0059 to 11×10^3 cels. mL^{-1} (BASU; PICK, 1997; CARLOUGH; MEYER, 1989; PICARD; LAIR, 2005; WEITERE; ARNDT, 2003) and between 0.49 and 54.6 μC L^{-1} (CARLOUGH; MEYER, 1989; JOAQUIM-JUSTO et al., 2006), respectively.

As observed for species composition and richness, none pattern of longitudinal distribution for density and biomass was verified. Only seasonal alterations of these attributes were observed, however, the density of autotrophic fraction presented significant differences among studied regions, with higher values at the middle course of the streams.

Although there have been significant differences in density of autotrophic fraction and also tendencies of higher values for the other attributes in the middle course of the streams, the results also lead to the rejection of the second hypothesis of the present study, since only these differences did not furnish bases sufficiently strong to infer that the regions of the streams supports qualitative and quantitative patterns distinct among them, although there is this tendency. Thus, it is evident that the temporal patterns (probably promoted by rainfall) were more important than space.

Based on Pearson correlations, we may infer that the temporal pattern observed for the abundance was mainly influenced by phosphate, nitrate, dissolved oxygen and water temperature, since the higher abundances were verified during the winter, concomitantly to higher values of phosphate, nitrate, dissolved oxygen and lower temperatures.

Several studies have shown that the increase in the nutrients availability causes the increase in density and biomass from flagellate community (GASOL et al., 1995; HWANG; HEALTH, 1997; WEISSE, 1991), because promotes the expansion of their food resources (bacteria and nanophytoplankton). Moreover, according to previously discussed, the water temperature and the oxygen availability are considered the main limiting factor for these organisms (ARNDT et al., 2000). In this way, the lower temperatures coupled to the higher availability of dissolved oxygen may have favored this increase in the abundance during the winter. Moreover, in the summer rainfall seems to break the stability of these systems and act as a disturbing agent that community, which had lower abundance and richness in this period.

Conclusion

In summary, the results showed that temporal patterns were more important than the space and probably regulated by rainfall, which breaks the stability these systems and therefore modifies the structure of this community (composition, richness and abundance). This absence of remarkable longitudinal patterns for attributes of protozoan community may be associated to the passive displacement of nanoflagellate along the course of the streams. However, it is extremely difficult to exactly diagnose which factors (active migration, passive displacement, predator presence and concentrations of food resource, among others) are responsible for the patterns of spatial distribution of this community, because they can act together. Finally, the results provide important information for monitoring programs in small-sized streams, since they show that to study plankton is not necessary a large sampling effort along the course of these streams, since there are no differences between regions.

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