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Protozoa ciliates community structure in urban streams and their environmental use as indicators

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Abstract

The objective of this work was to investigate the patterns of diversity and abundance of ciliates protozoa community in three tropical urban streams and verify which species can be considered bioindicators of water quality. In each stream, three regions (headwater, middle and mouth) were sampled in two years, in the rainy and dry seasons. The abiotic factors (dissolved oxygen, pH, temperature, turbidity, width, depth, flow and conductivity) and biotic factors (total coliforms, bacterioplankton, chlorophyll and flagellates) were analyzed using appropriate methods and ciliates were identified and counted through specialized literature. We identified 84 species distributed into 24 orders. Peniculida was the most representative order followed by Prorodontida. The RDA scores showed a spatial difference between streams. On the other hand, regarding the temporal variation, there was no separation of the sampled data. The Indval analysis showed ten species indicators, such as *Coleps hirtus*, *Euplotes moebiusi* and *Tintinidium pusillum*. The Cluster analysis per stream versus period showed a greater distinction of the streams in the dry season. On the other hand, a low similarity was observed between sections within the same stream. In summary, the results indicated that the ciliates can be used as important tools as bioindicators in lotic environments affected by different degrees of pollution, due to the fact that they have short life cycle, allowing the detection of impacts on a small time scale, as well as by having direct answers to environmental changes and high sensitivity to contaminants.

Keywords: protists, urban stream, diversity, abundance, trophic.

Estrutura da comunidade de protozoários ciliados em córregos urbanos e sua utilização como indicadores

Resumo

O objetivo deste trabalho foi investigar os padrões de diversidade e abundância da comunidade de protozoários ciliados em três córregos urbanos tropicais, bem como verificar quais espécies podem ser consideradas bioindicadoras da qualidade da água. Em cada córrego foram amostradas três regiões (cabeceira, intermediária e foz) em dois anos, nas estações chuvosa e seca. Os fatores abióticos (oxigênio dissolvido, pH, temperatura, turbidez, largura, profundidade, fluxo e condutividade) e fatores bióticos (coliformes totais, bacterioplâncton, clorofila e flagelados) foram analisados por metodologia específica e os ciliados foram contados e identificados por meio de literatura especializada. Foram identificadas 84 espécies distribuídas em 24 ordens. Peniculida foi a ordem mais representativa, seguida por Prorodontida. Os escores da RDA mostraram uma diferença espacial entre os córregos. Por outro lado, em relação à variação temporal, não houve separação entre os dados amostrados. A análise Indval mostrou dez espécies indicadoras, como *Coleps hirtus*, *Euplotes moebiusi* e *Tintinidium pusillum*. A análise de Cluster realizada por riacho versus período evidenciou uma maior distinção dos córregos no período seco. Por outro lado, foi observada uma baixa similaridade entre os locais dentro do mesmo córrego. Em síntese, os resultados indicaram que os ciliados podem ser utilizados como ferramentas importantes como bioindicadores em ambientes lóticos afetados por diferentes graus de poluição, devido ao fato de apresentarem um curto ciclo de vida, permitindo a detecção de impactos em uma pequena escala de tempo, bem como por apresentarem respostas diretas às mudanças ambientais e alta sensibilidade a contaminantes.

Palavras-chave: protistas, córregos urbanos, diversidade, abundância, trofia.

1. Introduction

The demand for freshwater has grown ominously worldwide, but the degradation of its quality has further reduced its availability (Kuhl et al., 2010). Rural and urban watersheds suffer from undue human activities, which alter the characteristics, the balance and the dynamics of natural resources making it difficult the supply of good quality water. Along with these changes, the increase in discharge of pollutant loads into water systems and urbanization usually results in the interruption or limitation of the use of these resources given the impacts on the quality of these ecosystems (Madoni, 2005).

Urban streams are usually subjected to degradation from high population density, with increased waterproofing, such as sidewalks, asphalt in street, avenues and roads. To monitor these impacts, both physical and chemical factors as the biotic variables have been used considering that they respond to environmental changes and the effects of pollution (Walsh et al., 2005). Thus, the integrated assessment of these factors allows us to evaluate the replenishment ability and sustainable use of resources, providing a systematic and integrated perception of environmental reality.

Ecological studies involving small and medium-sized streams including the fauna composition have increased in recent year. Such studies have focused on the diverse effects of human activities on these environments (Kühl et al., 2010; Camargo and Velho, 2011; Cunico et al., 2012).

Recent studies describe the effects of urbanization on streams, including hydrographic changes, rise in concentrations of nutrients and contaminants and changes in morphology and stability of the channel (Walsh et al., 2005; Cunico et al., 2012).

For a better understanding of a river water quality, data based on physical (temperature, turbidity, width, depth and flow), chemical (conductivity, pH, dissolved oxygen) and biological (algae, bacteria, fecal coliform and others) characteristics should be considered (Bere and Tundisi, 2010).

The use of biological communities as assessment tools is increasingly becoming important, because they are good indicators of the influence of human expansion on the environment, and are important in assessing water quality (Munn et al., 2002). Among the aquatic communities, protoplankton communities represent the first levels of trophic webs and essential in the transfer of matter and energy (Fenchel, 1987).

According to some studies, ciliates are efficient quality bioindicators of freshwater environments and can be used in biomonitoring of streams, lakes and reservoirs under different levels of anthropogenic impact (Paiva and Silva-Neto, 2004; Madoni, 2005; Dias et al., 2008). In this sense, these organisms have favorable characteristics, being a large group found in all aquatic environments (Zingel, 2005), with short life cycle and high reproduction rate, allowing the detection of environmental impacts in a short timescale (Groliére et al., 1990). Also, they are indicative of oxidizing or reducing conditions in the decomposition of organic matter, participating in phosphorus and nitrogen cycling, have high taxonomic density and are sensitive to different concentrations of pollutants, providing responses to different contamination levels (Paerl et al., 2003).

Studies on the species diversity patterns of these protozoa are complicated by methodological problems especially in regard to the species identification (Auer and Arndt, 2001). Consequently, there are few studies on the longitudinal variation in species diversity and density of protozoa ciliates particularly in urban streams (Madoni and Bassanini, 1999; Dias et al., 2008).

This study aims to investigate the patterns of diversity and abundance of the ciliate community and the factors involved in its structuring in three tropical urban streams, as well as to determine which species can be considered bioindicators of water quality.

2. Material and Methods

2.1. Study area and sampling design

The study area included three first-order urban streams, belonging to the upper Paraná River basin, sub-basins of the rivers São Francisco Verdadeiro and Piquiri (Figure 1). The São Francisco Verdadeiro River sub-basin drains about 10 municipalities in Western Paraná State, the municipalities of Cascavel and Toledo are the two largest urban centers in this region. The São Francisco Stream, in the municipality of Cascavel, is 2 km long, with banks occupied by residential areas and throughout its course, this stream receives drainage of surface waters from the surrounding neighborhoods. The Pinheirinho Stream, located in the municipality of Toledo, is affected by agricultural occupation in the surroundings, and is impacted by the residential areas, the introduction of species, pollutant input and other human activities. The Piquiri River basin crosses a region of intensive farming, and current urban development (Figure 1). The main use of this basin is the public water supply and a major problem is that only 10% of the people living in the cities located in the basin are served by sewage treatment (Gubiani et al., 2010).

The municipality of Palotina has about 30,000 inhabitants and various water bodies altered by urbanization, mainly related to lack of sanitation. An example of this is the Jequitibá Stream, which has approximately 3 km² drainage area and entire length in the urban area of the municipality, with land use consisting of the urban and rural areas, with residential areas, farming and animal husbandry (Gubiani et al., 2010).

Four samplings were conducted in two hydrological periods: rainy (April 2012 and 2013) and dry (September 2012 and February 2013) periods, to collect composite samples under the water surface, in three regions (headwaters, middle and mouth) of each stream.

2.2. Data collection/field sampling

For the rainfall of each sampling period, we considered the average of the five days prior to the date of each sampling. Data were provided by SIMEPAR - Paraná Meteorological Station, considering the months of April 2012 and 2013 as the rainy period and the months of September 2012 and February 2013 as the dry period.

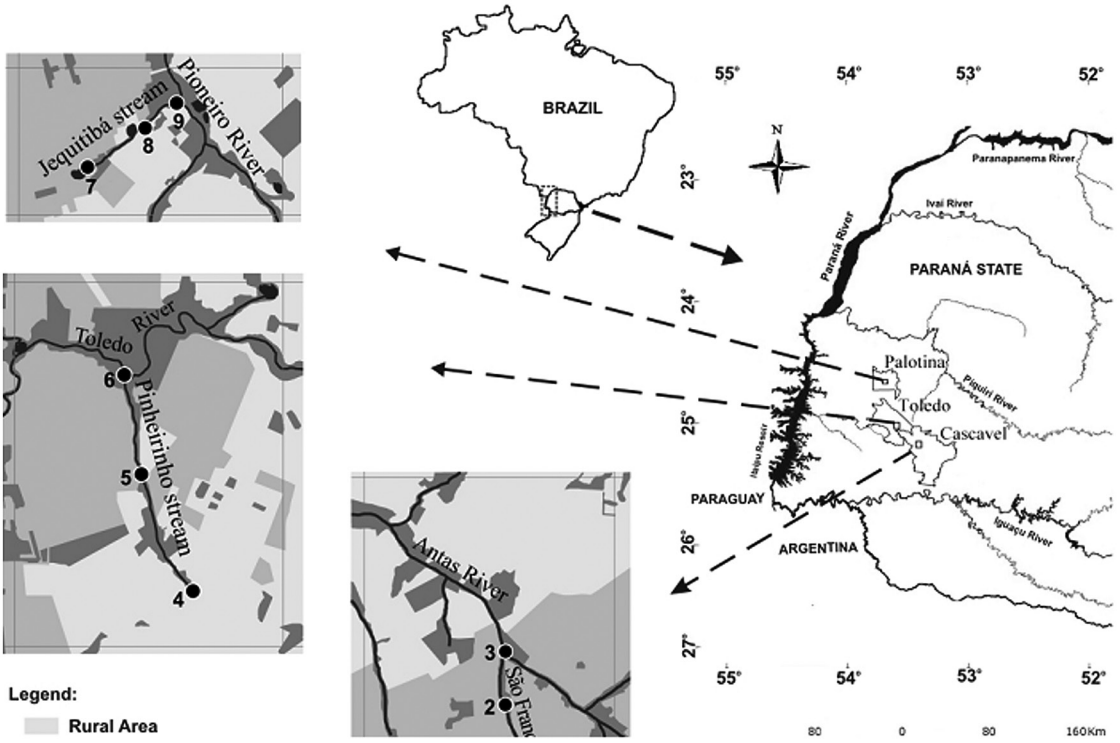

Figure 1. Sampling sites in three streams located in the cities of Palotina, Toledo, and Cascavel, state of Paraná, southern Brazil.

For microbial communities (ciliates, heterotrophic flagellates and heterotrophic bacteria) were collected five liters of water for each sample, with polyethylene plastic bottles, stored in coolers, and transported to the laboratory for analysis. Of these 5 liters, 4 liters were separated for the analysis of ciliates community. In turn, for the bacterioplankton and heterotrophic flagellates were reserved approximately 100 mL of the total water collected from each sample, that were fixed with a solution composed of formaldehyde buffered with borate, alkaline Lugol´s solution and sodium thiosulfate (Sherr and Sherr, 1993).

For determination of the fecal coliforms, samples were collected in sterile glass flasks and kept under refrigeration. The Multiple Tube technique were used (MPN) for this determination (APHA, 2006).

The electrical conductivity (µS/cm), dissolved oxygen (mg/L), pH, water temperature (°C), flow (m/s), depth (m) and width (m) were also measured with the help of portable devices and measuring tape. In situ, we collected 2 liters of water for analysis of chlorophyll-a (mg/L) and 10 mL of water for turbidity (NTU).

2.3. Laboratory analysis

2.3.1. Ciliates

Each ciliate sample were concentrated to 100 mL. Samples were examined in the laboratory and abundance ratios were determined within 5 h after collection. Estimates of ciliates species in each sample were made by direct observation in vivo under an optical microscope, at 100× and and 400× magnifications (Weisse, 1991), and counting 10 slides of 100 µL per sample (Madoni and Bassanini, 1999; Madoni, 2005). The total density was expressed as individuals/L. The identification was based on specialized literature (Foissner and Berger 1996; Foissner et al., 1999). The taxonomic classification was performed according to Lynn (2008).

2.3.2. Heterotrophic flagellates

Samples (100 mL) were fixed with a solution of formaldehyde, and alkaline lugol and thiosulfate (Sherr and Sherr, 1993). The density of heterotrophic flagellates was estimated by filtering subsamples of 10 mL through black Nucleopore Filters (0.8µm), stained with 1mL fluorochrome DAPI (4,6-diamidino-2-phenylindole) at 0.1%, for 15 minutes in the dark. Counting was performed in an epifluorescence microscope (Olympus BX51). To determine the flagellate density on each slide, we counted at least 50 fields under UV light, at 1000× magnification. The total density was expressed as individuals/L (Sherr and Sherr, 1993).

The differentiation of each fraction of flagellates was made simultaneously, using as a criterion the reddish color of autotrophs when subjected to blue light in contrast to the greenish color of heterotrophs, and only the data of heterotrophic flagellates were considered in this study.

2.3.3. Bacterioplankton

The process of fixation and estimation of density of the bacterioplankton were determined using the same protocol of filtration, mounting and storage of slides described for flagellates (Sherr and Sherr, 1993). However, subsamples of 1mL water were filtered through black Nucleopore/Watchman filter (0.2 µm) stained with 1 mL fluorochrome DAPI (4,6-diamidino-2-phenylindole) at 0.1%, for 5 minutes in the dark. Bacteria were quantified at 1000× magnification in an epifluorescence microscope (Sherr and Sherr, 1993). The total density was expressed in cells/mL.

2.3.4. Chlorophyll and turbidity

Concentrations of chlorophyll-a (µg/L) were determined at the Laboratory of Basic Limnology of Nupélia/UEM, according to Golterman et al. (1978).

The determination of turbidity was carried outat the LEPI - Laboratory of Fisheries and Ichthyology/UFPR - Campus Palotina, using a turbidimeter HACH 2100P (APHA, 2006).

2.3.5. Total coliforms

Thermotolerant coliforms were determined according to APHA (2006). The values of the analyzed characteristics were compared with the values recommended by CONAMA Resolution 357/2005 (Article 34) (Brasil, 2005), in order to check whether the parameters are within the acceptable standards by the aforementioned legislation.

2.4. Data analysis

2.4.1. Redundancy Analysis – RDA

We used Redundancy Analysis (RDA) (Legendre and Legendre, 1998) to estimate the relative role of environmental predictors (water temperature, dissolved oxygen, pH, electrical conductivity, turbidity, width, depth, chlorophyll, total coliforms and flagellates) on the structure of the ciliate community.

This analysis was applied to investigate the influence of environmental variables, as well as bacteria, chlorophyll and flagellates on the ciliate community structure. To this end, we used a matrix with the density of the different species of ciliates and another matrix with the abiotic variables (water temperature, pH, electrical conductivity, dissolved oxygen) and biotic variables (chlorophyll, density of bacterioplankton, total coliforms and flagellate). Biotic and abiotic data were previously log transformed except for pH. This analysis was performed using the software R 3.0.1, and the package vegan.

2.4.2. Indicator species

We calculated for each stream and period a Indicator Value Analysis (INDVAL - Dufrêne and Legendre 1997) to identify possible indicator species. This method determines the most characteristic organism for each environment and evaluates their specificity with a score ranging from 0 to 1, 1 being an organism present only in a given environment and totally absent from the others. This analysis was performed using the software PC-ORD (McCune and Mefford, 1999).

2.4.3. Cluster Analysis

The general patterns of similarity between the streams and sampling sites within the streams were evidenced by a Cluster analysis using the software PCOrd 8.6. For this analysis, we considered the presence and absence of species of ciliates at each sampling site in different hydrological periods (rainy and dry). The analysis used as a similarity coefficient the Unweighted Pair Group Method Average (UPGMA) linkage method, in which the distance between two groups is the arithmetic average of the distance between all pairs of objects belonging to both groups (Anderberg, 2014). In calculating the distance between the species (distance coefficient), we used a simple Euclidean distance for graphical representation through a dendrogram.

3. Results

3.1. Ciliate species composition

We identified 84 species distributed into 24 orders. Peniculida was the most representative order (12 species) followed by Prorodontida (8 species) (Table 1). The only taxa recorded in all samples of the three streams and in both hydrological periods was *Vorticella aquadulcis* complex (Order Sessilida).

3.2. Influence of environmental variables on ciliate communities

The first and second axis of the RDA were significant ($p<0.005$) and were retained for interpretation (R^2 adjusted= 0.40;$p=0.006$). The RDA analysis showed that the intervenient factors (biotic and abiotic variables) together with the most abundant ciliate species accounted for 40% of the data variability for the three streams in the rainy and dry periods (Figure 2).

RDA scores showed a spatial difference between streams (Figure 2). Thus, axis 1 separeted Jequitibá from São Francisco and Pinheirinho streams. As Jequitibá associated with most environmental variables, such as conductivity, dissolved oxygen, depth, pH, turbidity, temperature and flow, as well as flagellates heterotrophic density and chlorophyll concentration. While São Francisco and Pinheirinho streams were associated only with bacteria and total coliforms. Regarding the temporal variation, there was no separation of the sampled data.

Dissolved oxygen, conductivity and width along with chlorophyll and flagellates were positively associated to the distribution of the ciliates *Caenomorpha* sp., *Dexiostoma campylum*, *Lembadion bullinum, L. lucens, Paramecium caudatum, Tetrahymena piryformis* and *Tintinnidium pusillum.*

The abiotic variables flow, turbidity, temperature and pH influence the distribution of *Coleps hirtus, Cyclidium glaucoma, Entamoeba histolytica, Halteria grandinella, Monodinium balbiani, Ophryoglena* sp., *Dexiostoma tetraedricum, Urocentrum turbo, Vorticella convallaria* and *V. campanula* (Figure 2). *Aspidica cicada, Cinetochilum*

Table 1. Inventory of ciliated protozoa in three urbanized streams (Jequitibá, Pinheirinho and San Francisco) in each hydrological period where "x" indicates presence and "-" indicates absence of that species in that stream and period.

Order	Cod*	Jequitibá		Pinheirinho		São Francisco	
		Rainy	Dry	Rainy	Dry	Rainy	Dry
ARMOPHORIDA							
Caenomorpha sp.	*Caen. sp*	-	x	x	-	-	-
CHLAMYDODONTIDA							
Chilodonella uncinata (Ehrenberg, 1838)		-	x	-	x	-	x
CHOREOTRICHIDA							
Rimostrombidium humile (Penard, 1922)		x	-	-	x	-	-
Rimostrombidium lacustris (Foissner, Skogstad& Pratt, 1988)		-	x	-	x	-	x
COLPODIDA							
Colpoda cucullus Müller, 1773		-	x	-	-	-	x
Colpoda sp.		-	-	-	-	-	x
CYCLOTRICHIIDA							
Cyclotrichium viride Gajewskaja, 1933		-	x	-	-	-	x
Mesodinium pulex (Claparède &Lachmann, 1858)		x	-	-	-	-	-
EUPLOTIDA							
Aspidisca cicada (Müller 1786)	*A.cic*	x	-	-	x	x	x
Aspidisca lynceus (Müller, 1773)		-	-	-	-	x	-
Euplotes aediculatus Pierson, 1943		x	x	-	-	-	-
Euplotes eurystomus (Wrzesniowski, 1870)		-	-	x	-	-	-
Euplotes moebiusi Kahl, 1932	*E. moe*	-	x	-	-	-	-
HAPTORIDA							
Actinobolina radians (Stein, 1867)		x	-	-	-	-	-
Actinobolina vorax (Wenrich, 1929)		-	x	x	-	-	-
Lagynophrya acuminata Kahl, 1935	*L. acu*	x	-	-	-	-	-
Monodinum balbiani Fabre-Domergue, 1888	*M. bal*	-	x	-	-	x	-
Paradileptus elephantinus (Svec, 1897)		x	-	-	-	-	-
Balantidium pellucidum Eberhard, 1862		x	x	-	-	-	-
HETEROTRICHIDA							
Blepharisma lateritium Ehrenberg,1831		-	x	-	-	-	-
Spirostomum caudatum (Müller, 1786)		-	-	x	-	-	-
Spirostomum minus Roux, 1901		x	-	-	-	-	-
Stentor muelleri Ehrenberg, 1832		x	-	-	-	-	-
Stentor multiformis (Müller, 1786)		-	x	-	-	-	x
Stentor niger (Müller, 1773)	*S. niger*	x	-	-	-	x	-
LOXODIDA							
Loxodes rostrum (Mueller, 1773)		-	x	-	-	-	x
NASSULIDA							
Nassula picta Greeff, 1888		-	x	-	-	-	-
OPHRYOGLENIDA							
Ophryoglena sp.	*Oph. Sp*	-	x	-	x	x	-
ODONTOSTOMATIDA							
Saprodinium sp.		-	-	-	-	x	-

*Cod: Code of species used in Redundancy Analysis - RDA.

Table 1. Continued...

Order	Cod*	Jequitibá		Pinheirinho		São Francisco	
		Rainy	Dry	Rainy	Dry	Rainy	Dry
PENICULIDA							
Frontonia atra Ehrenberg, 1833		x	x	x	-	x	x
Frontonia acuminata Ehrenberg, 1833	*F. acu*	-	-	x	x	x	-
Frontonia angusta (Kahl, 1931)		-	-	x	-	-	-
Frontonia sp.		-	-	x	-	-	-
Disematostoma tetraedricum (Fauré-Fremiet, 1924)	*D. tet*	-	x	-	-	x	x
Lembadium bullinum (Perty, 1852)	*L. bul*	x	x	-	-	-	x
Lembadium lucens (Maskell 1887)	*L. luc*	-	x	x	-	-	x
Marituja pelagica Gajewskaja 1928		-	x	-	-	-	-
Paramecium aurelia complex	*P. aur*	-	-	x	-	x	-
Paramecium bursaria (Ehrenberg, 1831)	*P. bur*	-	-	x	-	-	x
Paramecium caudatum Ehrenberg, 1834	*P. caud*	x	x	-	x	-	x
Stokesia vernalis Wenrich, 1929		-	-	-	x	-	-
PLEURONEMATIDA							
Ctedoctema acanthocryptum Stokes, 1884		-	-	-	x	-	-
Cyclidium glaucoma Müller, 1773	*C. glau*	-	x	x	x	x	-
Cyclidium heptatricum Schewiakoff, 1893		-	-	-	-	x	-
PLEUROSTOMATIDA							
Acineria uncinata Tucolesco, 1962		-	-	-	-	-	x
Amphileptus procerus (Penard, 1922)		x	x	-	-	-	x
Amphileptus sp.		x	-	-	-	-	-
Litonotus fusidens (Kahl 1926)		x	x	-	-	-	-
Litonotus lamella (Müller,1773)		-	-	-	-	x	x
PHILASTERIDA							
Cinetochilum margaritacium (Ehrenberg, 1831)	*C. marg*	-	-	-	-	x	x
Loxocephalus sp.		-	-	x	x	-	-
Philasterides armatus (Kahl, 1926)	*P. arm*	-	-	-	-	-	x
Uronema nigricans (Müller 1786)		x	x	-	-	-	-
PRORODONTIDA							
Bursellopsis sp.		-	-	x	-	-	-
Coleps spetai Foissner, 1984		x	-	x	-	-	-
Coleps hirtus (Müller, 1786)	*C. hirt*	x	x	-	-	-	-
Holophyra discolor Ehrenberg, 1833		x	x	-	-	-	-
Prorodon ellipticus (Kahl, 1930)		-	x	-	-	-	-
Urotricha agilis (Stokes, 1886)		x	-	-	-	-	x
Urotricha globosa Schewiakoff, 1892		-	-	-	-	-	x
Urotricha sp.		-	-	-	-	-	x
SCUTICOCILIATIDA							
Dexiotrichides centralis (Stokes 1885)		-	-	-	x	-	-
SESSILIDA							
Astylozoon faurei Kahl, 1935		-	-	x	-	-	-
Campanella umbellaria (Linnaeus, 1758)		-	x	-	-	-	-
Vorticella aquadulcis complex	*V. aqua*	x	x	x	x	x	x
Vorticella campanula Ehrenberg, 1831	*V. camp*	x	x	x	-	-	-
Vorticella convallaria complex	*V. conv*	-	x	x	-	x	x
Vorticella spp.		-	-	x	-	x	x

*Cod: Code of species used in Redundancy Analysis - RDA.

Table 1. Continued...

Order	Cod*	Jequitibá		Pinheirinho		São Francisco	
		Rainy	Dry	Rainy	Dry	Rainy	Dry
SPORADOTRICHIDA							
Halteria grandinella (Müller, 1773)		-	x	-	-	x	-
Oxytricha haematoplasma Blatterer & Foissner, 1990		-	-	x	-	-	-
Oxytricha similis Engelmann, 1862		-	x	x	-	-	-
Pelagohalteria cirrifera (Kahl, 1932)		-	x	-	-	-	-
Stylonychia mytilus (Müller, 1773)		-	x	-	-	-	-
TETRAHYMENIDA							
Dexiostoma campylum (Stokes, 1886)	*D. cam*	-	x	-	-	x	x
Glaucoma scintillans Ehrenberg, 1830	*G. sci*	x	-	x	x	x	x
Glaucoma sp.		-	-	-	x	-	-
Tetrahymena pyriformis (Ehrenberg, 1830)	*T. pyr*	-	x	-	-	-	x
TINTINNIDA							
Codonella cratera (Leidy, 1877)		x	x	-	-	-	-
Tintinnidium pusillum Entz, 1909	*T. pus*	x	x	-	-	-	-
Tintinnidium sp.		x	-	-	-	-	-
UROCENTRIDA							
Urocentrum turbo (Müller, 1786)	*U. tur*	x	x	x	-	-	x
UROSTYLIDA							
Holosticha sp.		-	x	-	-	-	-
Holosticha kessleri Wrzesniowski, 1877		-	-	-	-	-	x
Uroleptus musculus Kahl, 1932		-	x	-	-	-	-

*Cod: Code of species used in Redundancy Analysis - RDA.

margaritaceum, Glaucoma scintilans and *Paramecium aurelia* were influenced by the biological variables total coliforms and bacterioplankton (Figure 2).

3.3. Indicator species

The results of the Indicator Value Analysis (INDVAL) revealed the occurrence of 10 species with bioindicator potential regardless of the period, especially *Coleps hirtus, Tintinidium pusilum* and *Euplotes moebiusi*, which had the highest levels of significance ($p < 0.024$, 0.019 and 0.018, respectively). The Jequitibá Stream had the largest number of bioindicator species, regardless of the hydrological period. On the other hand, the lowest number of bioindicator species was found in the Pinheirinho Stream (Table 2).

3.4. Cluster analysis

The Cluster analysis per stream versus period revealed a group formed by the streams Jequitibá in the dry period and São Francisco in both periods. The most distant group consisted of the streams Pinheirinho and Jequitibá in the dry period (Figure 3).

The Cluster analysis per stream section versus period evidenced a low similarity between sites within the same stream. The highest similarity occurred between JeqNC and PinFS, based on the species of ciliates. The most distant group was formed by PinMC and JeqMS (Figure 4).

4. Discussion

The species richness recorded in this study (88 taxa) is within the range found in different lotic environments. Studies on species richness of ciliates in lotic environments have shown an approximate range from 20 to 200 species of these protozoa (Madoni, 2005; Madoni and Braghiroli, 2007; Reiss and Schmid-Araya, 2008; Dias et al., 2008; Bradley et al., 2010; Pereira et al., 2014; Lobato Junior and Araújo, 2015).

The most species-rich orders were Peniculida and Prorodontida, which include algivorous, bacterivorous, omnivorous and predator species. These orders are especially found in mesotrophic and eutrophic environments, conditions prevalent in the studied streams (Kim et al., 2003; Gücker and Pusch, 2006; Eckert and Carrick, 2014).

On the other hand, some orders, like Oligotrichida, showed low species richness in the studied streams, maybe because the species of this order are more adapted to oligotrophic environments (Beaver and Crisman, 1989), and to planktonic life, requiring long residence time of the water to develop their populations (Pauleto et al., 2009).

The presence of the *Vorticella* in all samplings and with high abundances has great representativeness in various works developed in rivers and streams (Madoni and Braghiroli, 2007; Andrushchyshyn et al., 2007).

The species of this genus have preferably periphytic habit (Foissner and Berger, 1996), having the ability to

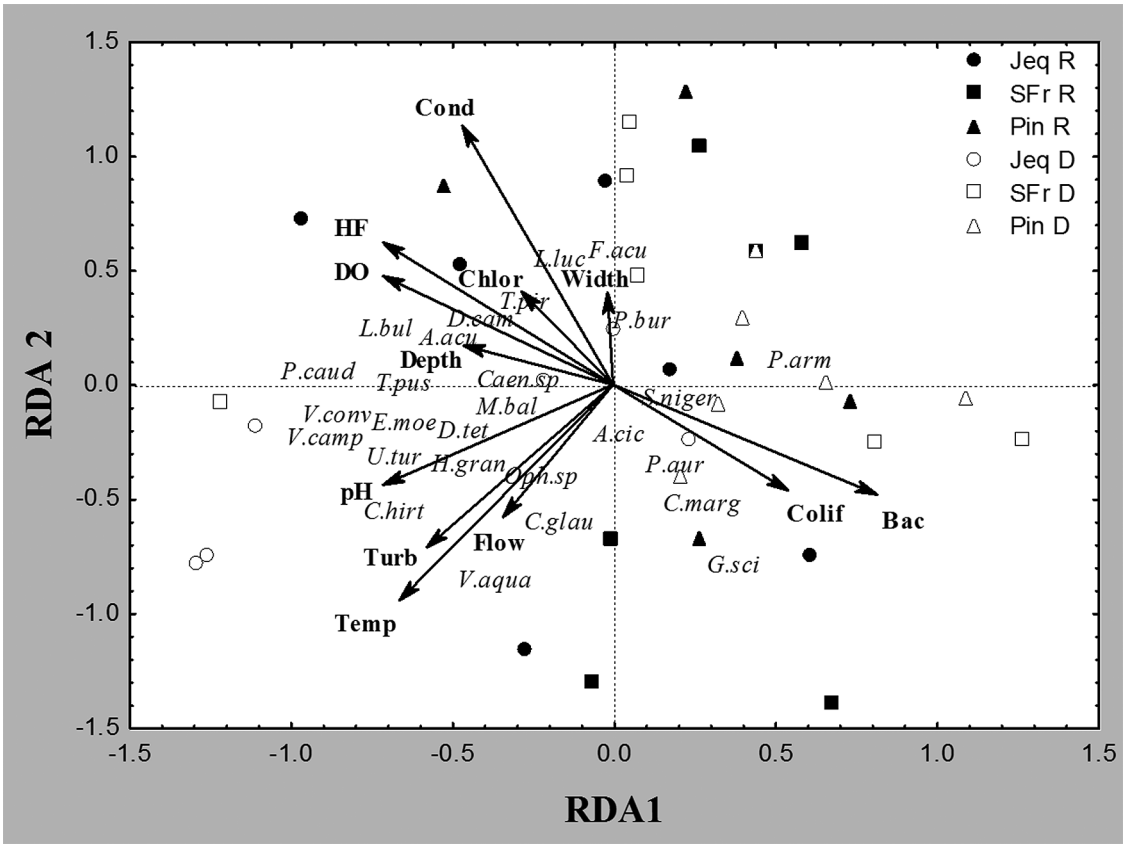

Figure 2. Ordination diagram for the first two axes of Redundancy Analysis, with the scores of streams sampled according to the abiotic variables: Cond=electrical conductivity; Temp=temperature; Turb= turbidity; DO=dissolved oxygen; Chlor= chlorophyll a; Bac= bacterioplankton; Colif: coliforms and HF= heterotrophic flagellates. Streams: JeqR: Jequitibá Rainy; JeqD: Jequitibá Dry; PinR: Pinheirinho Rainy; PinD: Pinheirinho Dry; SFrR: San Francisco Rainy and SFrD: San Francisco Rainy.

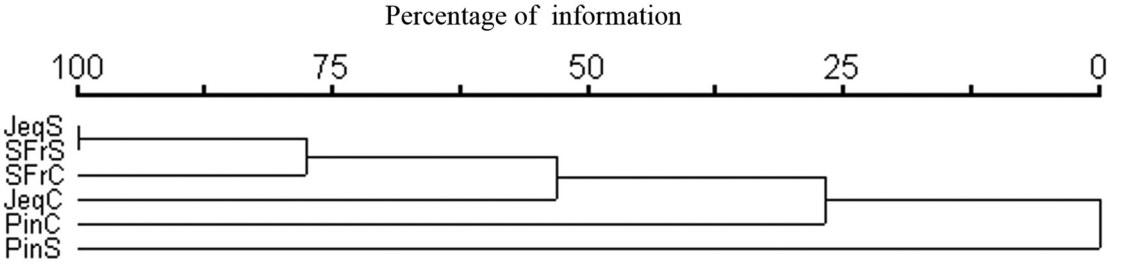

Figure 3. Dendogram Cluster analysis (UPGMA) showing the similarity between the streams and periods based on ciliates community. Jeq: Jequitibá; SFr: San Francisco; Pin: Pinheirinho; S: Dry; C: Rainy.

adhere to various substrates, making them more likely to resist mechanical action generated by the flow of lotic environments, therefore, considered as indicators of this type of environment (Kiss et al., 2009).

The high abundance of ciliates as *Caenomorpha* sp., *D. campylum*, *L. bullinum, L. lucens, P. caudatum, T. piryformis* and *T. pusilum* in environments with high concentrations of resources, such as chlorophyll and total coliforms, is probably related to their feeding habits, since these species primarily consume algae and bacteria (Zingel, 2005).

Furthermore, the positive relationship between the bacterivorous species *A. cicada, C. margaritaceum, G. scintilans* and *P. aurelia* and total coliforms and bacterioplankton, suggests that bacterial growth has been sufficient to maintain a high abundance of species with such feeding habits (Madoni, 2005).

The presence of some predatory ciliates, such as *D. campylum*, indicates the presence of flagellates in the

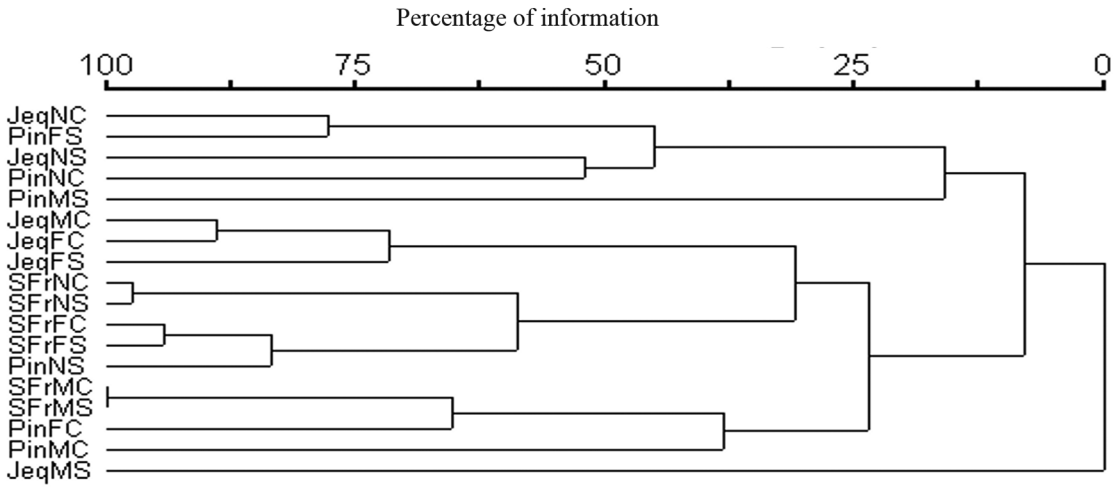

Figure 4. Dendogram Cluster analysis (UPGMA) showing the similarity between regions within each target stream and the hydrological period based on ciliates community. Jeq: Jequitibá; SFr: San Francisco; Pin: Pinheirinho; N: headwaters; M: middle; F: mouth; S: Dry; C: Rainy.

Table 2. Value of indicator species of ciliates in the Jequitibá, Pinheirinho and San Francisco streams in the rainy and dry seasons, derived from a IndVal analysis, based on occurrence data and abundance of species, where p <0.05. The values in bold type indicate that the content was significant.

Species	Jequitibá		Pinheirinho		São Francisco		*p*
	Rainy	Dry	Rainy	Dry	Rainy	Dry	
Acineria uncinata		X				X	0.084
Aspidisca cicada	X	X		X	X	X	0.067
Cinetochilum margaritacium					X	X	0.092
Codonella cratera	X	X					0.088
Coleps hirtus	X	X					**0.024**
Euplotes aediculatus	X	X					0.084
Euplotes moebiusi		X					**0.018**
Litonotus sp	X	X					0.090
Tintinidium pusillum	X	X					**0.019**
Vorticella campanula	X	X	X				0.051

sampled sites. According to Camargo and Velho (2011), the success of flagellate protozoa in streams can be attributed to human activities in the region, as some groups are considered indicators of impacted environments because of their preference for environments rich in organic matter (Reynolds and Descy, 1996).

The variables flow, turbidity, temperature and pH influence the distribution of some species of ciliates, because, depending on the amount and origin of suspended material, they can be favorable to the occurrence of bacterivorous, algivorous and omnivorous ciliates, such as *C. hirtus, C. glaucoma, E. histolytica, H. grandinella, M. balbiani, Ophryoglena. sp., D. tetraedricum, U. turbo, V. convallaria* and *V. campanula* (Foissner and Berger, 1996). Another important factor that may have contributed to the record of these species in these sites is the frequent physical disturbance of the bed during floods and anthropogenic changes nearby (Matthaei and Townsend, 2000), increasing or not the water flow in these environments, suggesting a continuous input of allochthonous organic matter into the investigated streams (Zingel, 2005). It is noteworthy that higher temperatures favor the consumption of bacteria, since bacterivory is temperature dependent and increases proportionally to this (Sherr et al., 1988). Regarding the influence of temperature and pH, Mansano et al. (2014) reported the highest densities of *C. glaucoma* and *H. grandinella* with higher values of pH and temperature.

None of the species indicated by the IndVal analysis is bioindicator of oligotrophic environments. This points out that the environments studied, although they are not under critical organic pollution, presented some species typical of beta-alpha-mesosaprobic, oligo-beta-saprobic and alpha-meso-saprobic environments (Foissner et al., 1995).

Besides, there was a greater similarity in ciliate species composition between streams than between sections within each stream. This may be due to different characteristics

in each section of the stream. Herein, the environments exhibited a low similarity to each other, with many rare species; only 26 species were recorded in more than two sampling sites, indicating a high turnover rate of species between sites.

This may be due to the conditions of each water body, as they are often disturbed by natural processes, such as changes in water flow and sediment input, and man-made activities, such as deforestation and removal of riparian vegetation, thus reducing the stability of the banks, increasing erosion and sedimentation processes, affecting in this way, the diversity of aquatic communities (Libório et al., 2013).

Although some sampled sites have been characterized by a relative load of organic pollution and some species have been indicator of these conditions, the community structure did not show the pattern expected for impacted environments, that is, anthropogenic changes reduce the richness of taxa to few generalist and tolerant species, leading to greater homogenization of biological communities, thereby decreasing diversity in streams (Rahel, 2002).

In this sense, the ciliate community distribution was mainly influenced by biotic and abiotic variables and not by seasonal conditions. There is greater similarity in ciliate species composition between streams than between sections within each stream. In general, the sites studied presented species associated with a certain trophic state, nevertheless, the community showed high diversity, indicating that these environments are not under severe pollution.

The results indicated that the ciliates can be used as important tools as bioindicators in lotic environments affected by different degrees of pollution, due to the fact that they have short life cycle, allowing the detection of impacts on a small time scale, as well as by having direct answers to environmental changes and high sensitivity to contaminants.

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