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Periphytic diatom as bioindicators in urban and rural streams

Carina Moresco* **and Liliana Rodrigues**

*Programa de Pós-graduação em Ecologia de Ambientes Aquáticos Continentais, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-230, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: camoresco@hotmail.com*

ABSTRACT. Periphytic diatoms were sampled from two streams, one located in an urban area and the other located in a rural area, in Maringá, State of Paraná, Brazil. In addition to temporal variability, differences in the assemblage structure between streams were evaluated and correlated with physical and chemical characteristics. Six samples of periphytic diatoms were collected from each stream from July 2007 to June 2008. The streams differed in abiotic factors and in relation to the structure of diatom assemblages and the spatial scale was more important than the temporal scale (ANOVA, $p \geq 0.05$; non-metric multidimensional scaling (NMS) stress = 13.73, $p = 0.009$). The Procrustes analysis ($m^2 = 0.7607$ and $p = 0.0001$) showed that variables with the greatest influence on the assemblage structure of diatoms were total nitrogen (TN), electrical conductivity, dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) and flow. These results indicate that abiotic variables modified by the land-use had greater influence on the community structure of diatoms than seasonal changes in abiotic variables.

Keywords: indicator species, nutrients, human impact, Bacillariophyta, periphyton.

Diatomáceas perifíticas como bioindicadores em córregos urbanos e rurais

RESUMO. Diatomáceas perifíticas foram amostradas em dois córregos, um localizado na zona urbana e outro na zona rural, situados em Maringá, Paraná, Brasil. Foram avaliadas as diferenças na estrutura da comunidade entre os córregos, relacionando-as às características físicas e químicas, bem como a variabilidade temporal. Seis amostras de diatomáceas perifíticas foram coletadas ao longo de cada córrego, de julho de 2007 a junho de 2008. Os córregos diferiram tanto em relação aos fatores abióticos como em relação à estrutura da comunidade de diatomáceas, e a escala espacial foi mais importante do que a escala temporal (ANOVA, $p \geq 0,05$; NMS – estresse = 13,73; $p = 0,009$). A análise de Procrustes ($m^2 = 0,7607$ e $p = 0,0001$) mostrou que as variáveis com maior influência sobre a estrutura da comunidade de diatomáceas nos córregos estudados foram o nitrogênio total (NT), condutividade, oxigênio dissolvido (OD), demanda química do oxigênio (DQO), demanda bioquímica de oxigênio (DBO_5) e velocidade da água. Os resultados indicam que as variáveis abióticas, alteradas pelo uso do solo, tiveram maior influência sobre a estrutura da comunidade de diatomáceas perifíticas do que a variação das condições abióticas devido às estações do ano.

Palavras-chave: espécies indicadoras, nutrientes, impacto antrópico, Bacillariophyta, perifíton.

Introduction

Over the last century, humans have caused drastic environmental changes through deforestation and the conversion of land for agricultural use (WINEMILLER et al., 2008). Urbanization stands out as a major modification to an ecosystem, by reducing soil permeability and causing hydrographic alterations, increasing nutrient concentrations and changing the morphology of waterways (WALSH et al., 2005).

With the increased human influence on hydrographic basins, the identification of factors that affect aquatic ecosystems and how these factors influence aquatic communities is a great challenge (PAN et al., 2004). The structure of aquatic communities is determined by processes that operate at multiple spatial scales, such as differences in habitat, current flow, nutrient availability and luminosity, which directly affect the organisms (TISON et al., 2005).

Biological communities in rivers and streams are important components in the evaluation of water quality (WHITTON; KELLY, 1995). Diatoms are important autotrophs in streams and rivers (STOERMER; SMOLL, 1999), and are sensitive to the physical and chemical variations of a body of water (WINTER; DUTHIE, 2000a and b). The composition and relative abundance of diatoms are determined by the preferences and tolerance of the species (LANGE-BERTALOT, 1979; VAN DAM et al., 1994; POTAPOVA; CHARLES, 2003). Variations in these attributes occurs in both the spatial and temporal scales and are ruled by differences in physical and chemical properties of water (STEVENSON; PAN, 1999), which has led to the growing use of diatoms in studies that monitor the water quality of rivers and streams (STOERMER; SMOLL, 1999; SOININEN et al., 2004). Variations may also be related to human activity in water basins, such as urbanization and agriculture (FORE; GRAFE, 2002).

Although several indices have been developed to evaluate the water quality using diatoms (VAN DAM et al., 1994; STEVENSON; PAN, 1999), only few studies have been conducted to assess the responses from diatoms along basins influenced by urbanization and by agricultural practices (LOBO et al., 1996; GÓMEZ; LICURSI, 2001; JÜTTNER et al., 2003), especially in tropical regions. In Brazil, bioindication studies of water quality using diatoms were developed from the 80's, mainly in South and Southeastern regions, focusing on lotic environments (LOBO; TORGAN, 1988; LOBO et al., 1996, 2002, 2004a, b and c; LOBO; CALLEGARO, 2000; BURLIGA et al., 2004; HERMANY et al., 2006; SALOMONI et al., 2006; DÜPONT et al., 2007, among others).

The objectives of this study were: i) to evaluate differences in the structure of periphytic diatom assemblages of streams from an urban and an rural watersheds and to correlate the differences in the assemblages with physical and chemical variables; ii) to analyze the temporal variability in the periphytic diatom assemblage in these streams; and iii) to select indicator species in each environment. Therefore, the following hypotheses were tested: a) the chemical influence of water on assemblages of periphytic diatoms in an urban environment is greater than on assemblages from a rural environment; b) the spatial variation of the assemblage of periphytic diatoms (urban and rural streams) is more important than the temporal variation (month of sampling).

Material and methods

Study area

The Pirapó river basin is located in the northeast corner of Paraná State, within the upper Paraná river system. The region has a drainage area of approximately 5,076 km^2, situated in the Serra Geral Formation, which is composed of basic igneous rocks, such as basalt (MINEROPAR, 2006), and a predominance of red nitosols. Waters from this basin are used for supply, development of agricultural activities and ecological tourism for most of the cities in this region. One city that receives the water from this basin is Maringá that is on the water divide of the Pirapó river basin and the Ivaí river basin, and the headwaters of several streams are located within the urban perimeter of Maringá.

The Nazaré Stream micro-basin has a drainage area of 867.928 hectares, is located in the urban area of Maringá (Figure 1) and contains 34.8% impervious surfaces, which are mostly located in residential and industrial areas. In this micro-basin, the main industrial activities are related to metallurgy, plastic and petroleum products (KÜHL et al., 2010). The banks are steep throughout its length. At the mouth and at the intermediate region, riparian forest is present on the right bank. At the headwater, the riparian forest is more developed and is present on both banks.

The Remo Stream micro-basin has a drainage area of 792.325 hectares, is located in the rural area of the municipality of Maringá (Figure 1) and has 0.5% impervious surfaces. Farming practices are the main activities performed in this micro-basin (crop rotation: maize, soybean and wheat). The Remo Stream has riparian vegetation on both banks along its course.

Abiotic variables

The sampling to determine the abiotic variables was performed concurrently with the sampling of biotic variables. Data of physical and chemical conditions of water, such as pH (DIGIMED DM2), electrical conductivity (DIGIMED DM3, μS cm^{-1}), dissolved oxygen, water temperature (YSI 55/12FT, ml L^{-1} and °C, respectively) and flow (FLO-MATE 2000, Marsh McBirdey, m s^{-1}) were all measured in the field with portable analytical equipment. The total nitrogen concentration, orthophosphate, biochemical oxygen demand (BOD$_5$, mg O$_2$ L^{-1}), chemical oxygen demand (COD, mg O$_2$ L^{-1}) and oil (mg L^{-1}) in water samples were analyzed by technicians from the Sanitation and Agrochemistry Laboratories from the State University of Maringá.

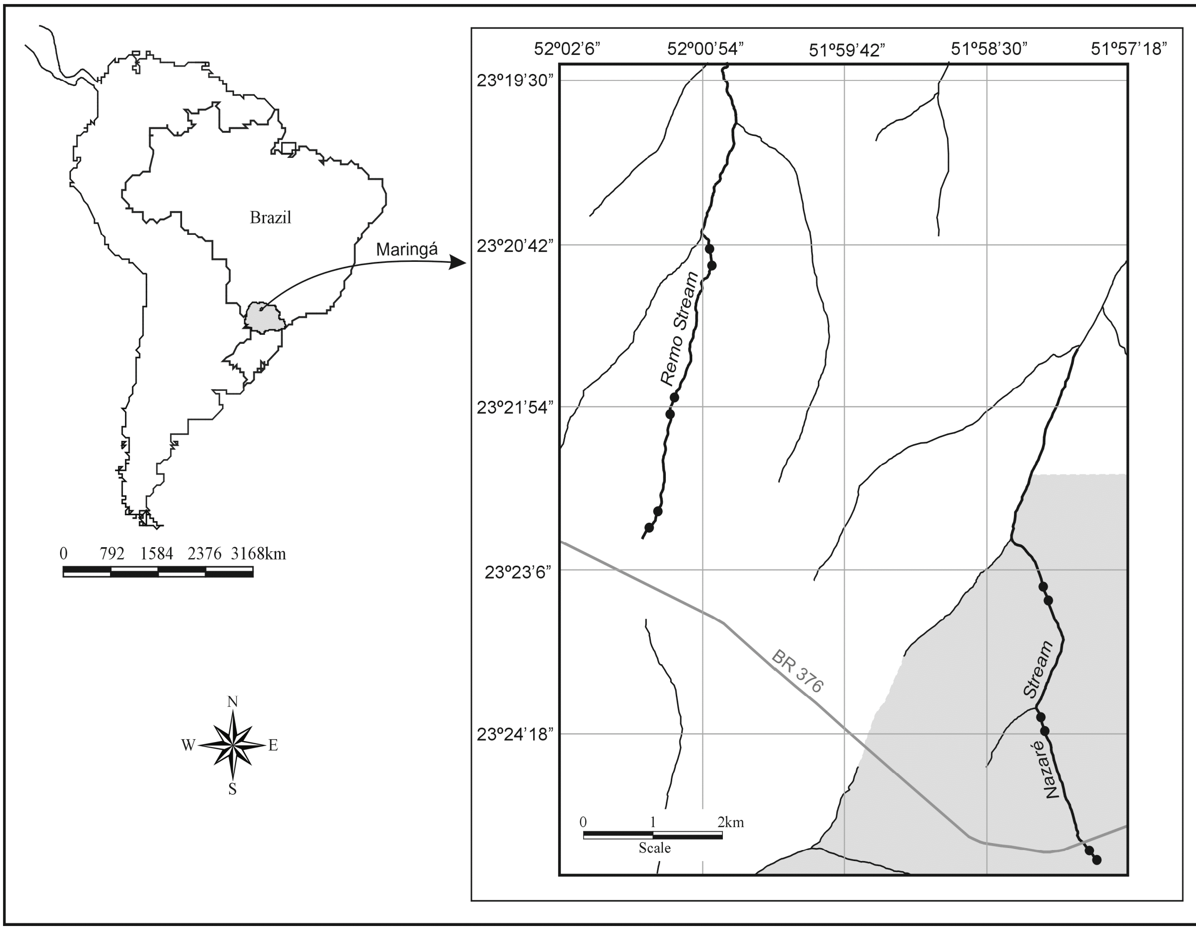

Figure 1. Location of the urban stream and the rural stream in Maringá Municipality (2007-2008). White: rural area and grey: urban area.

Sampling and collection of diatoms

Six samples of periphytic diatoms were collected from each stream every two months from July 2007 to June 2008. Each sample consisted of three pebbles (composite sample). This substrate was selected because it was the most abundant substrate and was present in both streams. The side of the rock opposite to the direction of the water current was scraped with the aid of a brush and a blade, and the material was fixed with 4% formalin (1:1 ratio). Measurements of the shaved surface area were taken with a caliper. The material was oxidized and Hyrax was used to mount the permanent slides. Slides with control material were deposited in the Herbarium of the State University of Maringá, in Maringá, Paraná State (HUEM).

Identification and counting of the diatom species was performed using a light microscope (Olympus CX31). Individuals were identified and counted until recording a minimum of 600 valves, as recommended by Kobayasi and Mayama (1982), and a 90% counting efficiency was used in accordance with Pappas and Stoermer (1996). The concentration of cells.cm^{-2} was estimated by multiplying the number of valves from each taxon by a conversion factor according to the methods of Hermany et al. (2006).

To identify the periphytic diatom taxa, the following taxonomic texts were used: Krammer and Lange-Bertalot (1991a and b), Rumrich et al. (2000), Lange-Bertalot (2001), Krammer (2002), Metzeltin and Lange-Bertalot (1998, 2007), Metzeltin et al. (2005) and Tremarin et al. (2009, 2010).

Data analysis

To identify possible significant spatial and temporal differences between the means of the attributes (richness, evenness, Shannon-Wiener diversity index and density) from the periphytic diatom assemblages, analysis of variance (ANOVA) was used, and the temporal variability was controlled through blocks (ANOVA; period factor: block; Local factor: rural and urban).

Assumptions of normality and homoscedasticity were checked using Shapiro-Wilk and Levene tests, respectively. When the assumptions were not met, data were log transformed. When the ANOVA was significant, a Tukey's test was employed to determine the level that has varied.

In order to summarize the assemblage structure of periphytic algae, a non-metric multidimensional scaling (NMS) was used (KRUSKAL, 1964). The Sørensen distance was calculated, and the general procedure for NMS was followed according to previously published methods (MCCUNE; GRACE, 2002). One-hundred permutations were performed, and the standard deviation was used as the stability criterion (≤ 0.005, stress above 100 interactions). This analysis was run with the matrix of abundance data (log transformed to remove the effect of high values) in different sampling locations and periods.

For testing significant differences between each location and period (summarized by NMS), this study used a multi-response permutation procedure (MRPP), which is a non-parametric method used to test for multivariate differences between predefined groups (ZIMMERMAN et al., 1985). The significance of the null hypothesis that the locations and periods were not different was tested by Monte Carlo randomization (based on 10,000 permutations).

To determine indicator species (IndVal), the methods presented by Dufrêne and Legendre (1997) were followed, by which the abundance and frequency of occurrence of the species in each group was used as the input data and indicator values calculated for each species (MCCUNE; GRACE, 2002). The species with $p < 0.05$ (based on 10,000 permutations) from the Monte Carlo test were considered to be an indicator species.

Environmental variables were summarized by a principal component analysis (PCA). To determine the components that should be retained for interpretation, the Broken Stick criterion was employed. According to this model, only axes with an eigenvalue greater than the eigenvalues generated by randomization should be interpreted (MCCUNE; GRACE, 2002). The abiotic data, except pH, were log transformed for PCA.

Relationships between the multivariate analyses (environmental variables and structure of periphytic diatom assemblage) were examined by a Procrustes analysis (PERES-NETO; JACKSON, 2001). In this analysis, the two matrices are compared using a logarithm that minimizes the residual sum of squares between two matrices (ROHLF; SLICE, 1990). The resulting value of m^2 is the best fit, which assumes that it describes the degree of association between the matrices.

NMS, MRPP, IndVal and PCA analyses were performed using the program PC-Ord® 4.0 (MCCUNE; MEFFORD, 1999). Procrustes statistics were calculated with the PROTEST® program (JACKSON, 1995). The ANOVA was computed using the software Statistica™ 7.0. The level of significance was $p < 0.05$.

Results

Table 1 shows the variation of physical and chemical variables of the studied streams. Water temperature and pH had no expressive differences between the urban and rural streams. Conductivity and total nitrogen presented higher values in the urban stream. Considering the orthophosphate, higher concentrations were observed in the urban stream, except in periods 1 and 2. In periods 3 and 6, values of this variable were similar between streams. Concentrations of dissolved organic carbon were greater in the rural stream, except in periods 4 and 5. Values of biochemical oxygen demand were also higher in the rural stream, with exception of periods 4 and 6. For oxygen and flow, values were slightly higher in the rural stream.

PCA has determined that 53.3% total variability of the abiotic data was in the first two axes (Table 2). In PCA 1 (30.63%), there was a clear separation between the streams. There were positive correlations with dissolved oxygen, chemical oxygen demand, biochemical oxygen demand and flow. A negative correlation was detected with conductivity and total nitrogen. In PCA 2 (22.67%), there were fluctuations in abiotic factors along the sampled periods, especially at the urban stream (Table 2 and Figure 2). The positively correlated variables were pH and dissolved oxygen, while water temperature, orthophosphate, chemical oxygen demand and biochemical oxygen demand showed a negative correlation.

Table 1. Mean and standard deviation of abiotic variables from the urban (U) and rural (R) streams. Sampling period 1 (July/07), 2 (September/07), 3 (December/07), 4 (February/08), 5 (April/08) and 6 (May-June/08). T = water temperature (ºC), pH = potential of hydrogen, Cond. = conductivity (μS cm^{-1}), DO = dissolved oxygen (mL L^{-1}), PO_4 = orthophosphate (mg L^{-1}), TN = total nitrogen (mg L^{-1}), COD = chemical oxygen demand (mg L^{-1}), BDO_5 = biochemical oxygen demand (mg L^{-1}) and flow (m s^{-1}).

	T	pH	Cond.	DO	PO_4	TN	COD	BOD_5	Flow
R1	16.2 (± 0.8)	6.5 (± 0.1)	105.6 (± 8.7)	8.7 (± 0.32)	123.3 (± 101.1)	7466.6 (± 2138.5)	10.9 (± 14.0)	1.34 (± 1.23)	0.22 (± 0.03)
R2	20.4 (± 0.3)	7.4 (± 0.2)	116.5 (± 6.6)	8.2 (± 0.44)	86.6 (± 45.1)	733.3 (± 404.1)	5.46 (± 4.68)	1.83 (± 1.02)	0.18 (± 0.05)
R3	21.9 (± 0.7)	7.1 (± 0.1)	112.7 (± 4.7)	7.8 (± 0.29)	93.3 (± 23.1)	1633.3 (± 230.9)	3.65 (± 2.51)	1.17 (± 1.04)	0.21 (± 0.05)
R4	21.5 (± 0.4)	7.0 (± 0.1)	119.2 (± 5.7)	8.4 (± 0.35)	50 (± 10)	1566.6 (± 208.1)	5.96 (± 2.50)	1.93 (± 0.66)	0.24 (± 0.07)
R5	20.9 (± 0.5)	7.0 (± 0.2)	125 (± 5.2)	8.7 (± 0.25)	62.6 (± 7.6)	1700 (± 200)	3.1 (± 0.55)	1.53 (± 0.05)	0.19 (± 0.04)
R6	17.6 (± 0.6)	6.8 (± 0.1)	119 (± 5.6)	8.1 (± 0.5)	80 (± 26.4)	1800 (± 200)	3.4 (± 1.45)	0.93 (± 0.05)	0.20 (± 0.04)
U1	15.3 (± 0.2)	7.5 (± 0.1)	264.6 (± 46.5)	8.5 (± 0.47)	43.33 (± 20.8)	7466.6 (± 808.2)	0.48 (± 0.25)	0.17 (± 0.04)	0.19 (± 0.04)
U2	18.3 (± 0.2)	7.4 (± 0.1)	294.3 (± 57.4)	8.1 (± 0.62)	30 (± 17.3)	5600 (± 3803.9)	1.36 (± 0.61)	0.7 (± 0.43)	0.12 (± 0.04)
U3	22.4 (± 0.1)	7.1 (± 0.1)	282.6 (± 55.4)	7.6 (± 1.14)	96.6 (± 40.4)	6933.3 (± 2936.5)	1.08 (± 0.62)	0.09 (± 0.03)	0.13 (± 0.03)
U4	21.6 (± 0.2)	7.0 (± 0.1)	310.3 (± 70.5)	7.9 (± 0.29)	116.6 (± 73.7)	7866.6 (± 3499.0)	7.3 (± 6.11)	2.53 (± 2.57)	0.16 (± 0.04)
U5	20.8 (± 0.2)	7.0 (± 0.1)	297.6 (± 59.7)	6.9 (± 0.64)	351 (± 513.7)	8100 (± 3143.2)	3.6 (± 0.55)	1.1 (± 0.1)	0.17 (± 0.04)
U6	18.4 (± 0.3)	6.8 (± 0.1)	282.6 (± 58.2)	6.9 (± 0.42)	86.6 (± 30.5)	8433.3 (± 3197.3)	2.83 (± 1.40)	1.5 (± 0.45)	0.15 (± 0.06)

Table 2. Results from the principal component analysis (PCA) using the matrix of abiotic variables sampled from July/2007 to May-June/2008 in urban and rural streams.

	Axis 1	Axis 2	Axis 3
Eigenvalues	3.063	2.267	1.429
Broken-stick	2.929	1.928	1.391
% of variance	30.63	22.673	12.907
T (ºC)	0.0057	-0.3161	-0.6486
pH	-0.0886	0.3503	-0.3459
Cond. (μS^{-1} cm^{-1})	-0.4954	-0.0973	0.1192
DO (mg L^{-1})	0.3535	0.3823	0.0332
PO_4 (mg L^{-1})	-0.0546	0.3823	0.0332
TN (mg L^{-1})	-0.4041	-0.1062	0.5012
COD (mg L^{-1})	0.3511	-0.4581	0.1154
BOD_5 (mg L^{-1})	0.3184	-0.4308	0.0501
Oil (mg L^{-1})	-0.2744	-0.1622	-0.376
Flow (m s^{-1})	0.3944	0.0936	0.1717

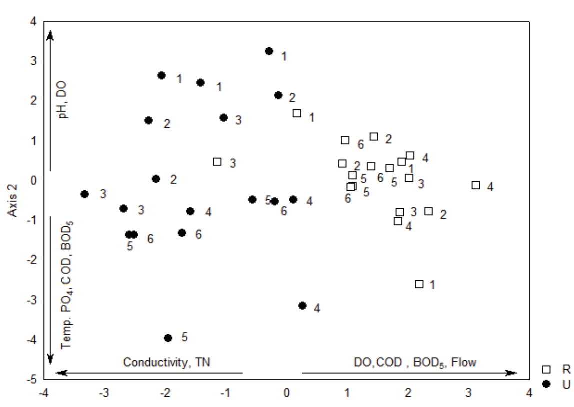

Figure 2. Spatiotemporal position of the urban (U) and rural (R) streams ordinated according to the first two axes from the principal component analysis; Cond. = conductivity (μS cm^{-1}), TN = total nitrogen (mg L^{-1}), DO = dissolved oxygen (mL L^{-1}), COD = chemical oxygen demand (mg L^{-1}), BOD_5 = biochemical oxygen demand (mg L^{-1}), flow (m·s^{-1}), T = water temperature (ºC), PO_4 = orthophosphate (mg L^{-1}) and pH = potential of hydrogen. Sampling periods: 1 (July/07), 2 (September/07), 3 (December/07), 4 (February/08), 5 (April/08) and 6 (May-June/08).

In total, 135 species were identified, and 69 taxa were common to both streams. One hundred and twenty four were detected in the rural stream, being 55 exclusive in this stream. Eighty taxa were observed in the urban stream and 11 species were exclusively found in this stream. Significant differences were detected for species richness between the streams (ANOVA, F = 28.92; p = 0.000009; Figure 3a), however, there was no significant difference in the time scale for this attribute (F = 0.4523; p = 0.80; Figure 3b).

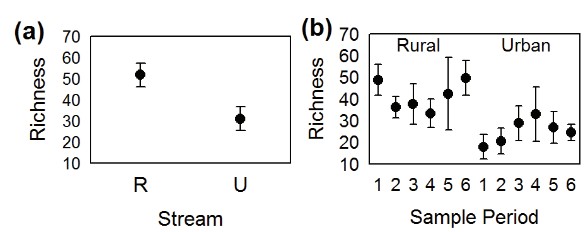

Figure 3. Variation (mean and standard deviation) in the number of periphytic diatom taxa in the rural (R) and urban (U) streams. Sampling periods: 1 (July/07), 2 (September/07), 3 (December/07), 4 (February/08), 5 (April/08) and 6 (May-June/08). Note scale.

The higher mean evenness was recorded in the rural stream (ANOVA, F = 5.274; p = 0.02; Figure 4a). However, no significant difference was found in evenness on the time scale (F = 0.519; p = 0.75; Figure 4b).

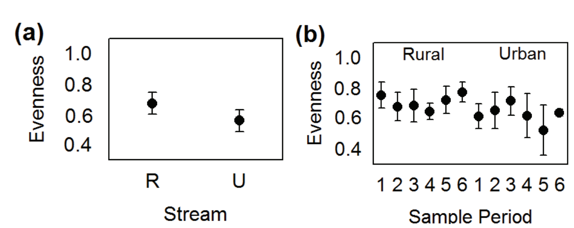

Figure 4. Variation (mean and standard deviation) in the evenness (%) of periphytic diatom assemblages in the rural (R) and urban (U) streams. Sampling periods: 1 (July/07), 2 (September/07), 3 (December/07), 4 (February/08), 5 (April/08) and 6 (May-June/08). Note scale.

The Shannon-Wiener diversity index had higher values in the rural stream (ANOVA, F = 12.38; p = 0.001; Figure 5a); however, there was no significant difference in diversity between the sampled periods (F = 0.38; p = 0.85; Figure 5b).

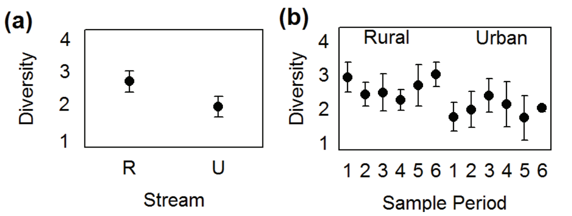

Figure 5. Variation (mean and standard deviation) in the Shannon-Wiener diversity index (bits cm^{-2}) of periphytic diatom assemblages in the rural (R) and urban (U) streams. Sampling periods: 1 (July/07), 2 (September/07), 3 (December/07), 4 (February/08), 5 (April/08) and 6 (May-June/08). Note scale.

The highest mean values of density were observed in the urban stream (ANOVA, F = 13.002; p = 0.001; Figure 6a); but no significant difference in density was registered on the time scale (F = 1.28; p = 0.29; Figure 6b).

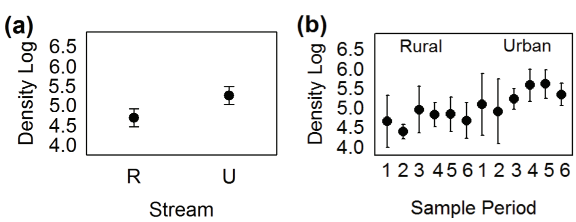

Figure 6. Mean variation in density (Log ind. x cm^{-2}) of periphytic diatom assemblages in the rural (R) and urban (U) streams. Sampling periods: 1 (July/07), 2 (September/07), 3 (December/07), 4 (February/08), 5 (April/08) and 6 (May-June/08). Note scale.

The structure of periphytic diatom assemblages summarized by a NMS showed a difference between locations and periods (Figure 7). After 31 interactions, the stability criterion was achieved with a final stress of 13.73 (Monte Carlo test, p = 0.009), and three axes were retained for interpretation. The proportion of variance represented by each axis, which was based on the distance between r^2 within the ordination space and distances in the original space, was 0.512 for axis 1, 0.158 for axis 2 and 0.218 for axis 3, all totaling 0.888.

The spatial scale was identified as the main pattern of the structure of the periphytic diatom assemblages, which was determined by plotting axes 1 and 2 (Figure 7a), axes 1 and 3 (Figure 7b) and axes 2 and 3 (Figure 7c), corroborating the result of the MRPP (p = 0.00000). The formation of a group for the rural stream and another group for the urban stream was related to the increased conductivity and total nitrogen values recorded for the urban stream, and to higher dissolved oxygen values, COD, BOD_5 and flow in the rural stream. These results are confirmed by the matrix correlation test (Procrustes) between the first three axes of PCA ordination, and the first three axes of NMS ordination. The adjusted value for the distribution of periphytic diatoms was m^2 = 0.7607 and p = 0.0001, which supported statistically the influence of abiotic variables on the spatial and temporal distribution of the assemblages in the urban and rural streams.

A clear temporal variability was not observed for attributes analyzed separately (Figures 3b, 4b, 5b and 6b). But when considered the community structure (richness and density simultaneously) a significant difference was detected (Figures 7a, b and c - MRPP, p = 0.0006).

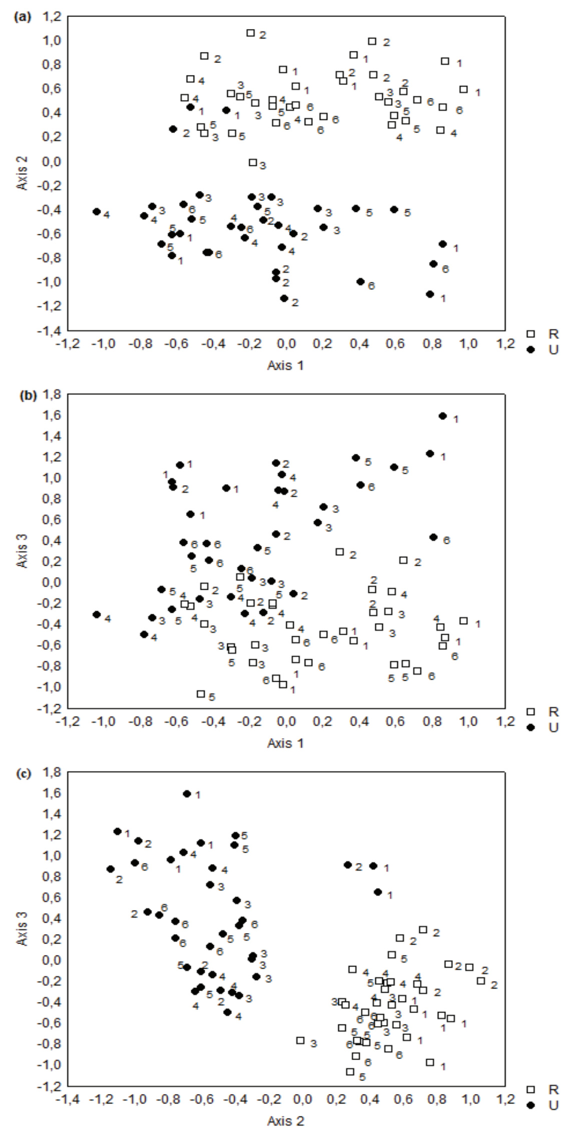

Figure 7. Scatterplots of non-metric multidimensional scaling (NMS) ordination performed on periphytic diatom assemblages from the rural (R) and urban (U) streams. Sampling periods: 1 (July/07), 2 (September/07), 3 (December/07), 4 (February/08), 5 (April/08) and 6 (May-June/08).

The analysis of indicator species (IndVal) has identified diatom species strongly associated with urban and rural streams (IndVal, p ≤ 0.05; Table 3). Thirty-two taxa were indicators in the rural stream, and 17 taxa were indicators in the urban stream.

Table 3. Indicator Species Analysis (IndVal) with indicator values (IV). Only statistical significant results (p < 0.05 for the Monte Carlo test) are shown.

	IV		Monte Carlo
	Rural	Urban	p
Achnanthes exigua Grunow	70	13	0.0066
Achnanthes rupestoides Hohn	78	15	0.0008
Amphipleura lindheimeri Grunow	89	0	0.0001
Amphora copulata (Kützing) Schoeman and Archibald	89	0	0.0001
Diploneis subovalis Cleve	81	0	0.0001
Encyonema mesianum (Cholnoky) Mann	65	0	0.0002
Fallacia ecuadoriana Lange-Bertalot and Rumrich	44	2	0.0359
Fallacia insociabilis (Krasske) Mann	67	0	0.0001
Fragilaria rumpens (Kützing) Carlson	39	0	0.0069
Frustulia crassinervia (Brébisson) Lange-Bertalot and Krammer	28	0	0.0473
Frustulia vulgaris (Thwaites) De Toni	46	2	0.0094
Geissleria neosubtropica Metzeltin, Lange-Bertalot and García-Rodriguez	56	0	0.0002
Gomphonema brasiliense Grunow	78	0	0.0001
Gyrosigma acuminatum (Kützing) Rabenhorst	59	0	0.0008
Gyrosigma scalproides (Rabenhorst) Cleve	42	0	0.0078
Hantzschia amphioxys (Ehrenberg) Grunow	28	0	0.0486
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metelzin & Witkowski	28	0	0.0462
Luticola dapalis (Frenguelli) Mann	48	0	0.0034
Navicula cryptotenella Lange-Bertalot	67	24	0.0185
Navicula lohmannii Lange-Bertalot & Rumrich	89	4	0.0001
Nupela praecipua (Reichardt) Reichardt	83	8	0.0002
Pinnularia sp.	28	0	0.0442
Placoneis constans var. *symmetrica* (Hustedt) Kobayasi	56	0	0.0006
Placoneis disparilis (Hustedt) Metelzin & Krammer	49	0	0.0018
Placoneis hambergii (Hustedt) Bruder	49	9	0.0297
Sellaphora pupula (Kützing) Mereschkowsky	79	5	0.0002
Sellaphora sp. 1	61	0	0.0001
Sellaphora sp. 2	76	0	0.0001
Stauroneis cf. *kriegeri* Patrick	64	1	0.0003
Stenopterobia schweickerdtii (Cholnoky) Brassac, Ludwig & Torgan	56	0	0.0004
Tryblionella levidensis Smith	79	1	0.0001
Ulnaria ulna (Nitzsch) Compère	56	11	0.0245
Sellaphora seminulum (Grunow) Mann	8	92	0.0001
Achnanthes lanceolata (Brébisson ex Kützing) Grunow	9	91	0.0001
Achnanthidium minutissimum (Kützing) Czarnecki	2	91	0.0003
Craticula sp.	0	89	0.0001
Nupela sp.	13	87	0.0018
Fallacia monoculata (Hustedt) Mann	0	83	0.0001
Gomphonema lagenula Kützing	19	81	0.0415
Amphora montana Krasske	1	77	0.0063
Eolimna minima (Grunow) Lange-Bertalot	27	73	0.0375
Navicula tenelloides Hustedt	6	69	0.0153
Navicula sp. 5	1	69	0.0008
Caloneis bacillum (Grunow) Cleve	0	66	0.0007
Pinnularia gibba (Ehrenberg) Ehrenberg	1	53	0.0292
Mayamaea atomus (Kützing) Lange-Bertalot var. *permitis*	0	50	0.0028
Cyclotella meneghiniana Kützing	0	39	0.0129
Nitzschia cf. *inconspicua* Grunow	0	28	0.0454
Sellaphora sp.	0	28	0.0444

Discussion

The assemblages of the periphytic diatoms studied were different between the urban stream and the rural stream. Analyses performed using attributes from this assemblage, such as richness, evenness, diversity and total density, indicated that the spatial variation (urban and rural streams) was more significant than temporal variation (month of sampling).

Studies on streams that drain urban areas have shown a decline in the richness of diatom species, and the reduction in species richness is associated with organic pollution (SONNEMAN et al., 2001). Common effects from pollution are the reduction of species diversity, increased density and an increase of tolerant species (JÜTTNER et al., 2003; NDIRITU et al., 2006). In addition, under conditions of intermediate nutrient concentrations, the periphytic diatoms may have increased diversity (JÜTTNER et al., 2003). In this study, higher concentrations of nutrients in the urban stream have affected periphytic diatoms. This stream presented a high density, but attributes such as richness, evenness and diversity were reduced, owing nutrient enrichment and human disturbance. In contrast, richness had higher values in the rural stream when compared to those recorded for the urban stream.

Studies that involve periphytic diatom assemblages have reported the influence of urban and rural environments on the structure and distribution of these assemblages. Winter and Duthie (1998) observed that the differentiation between streams basin were related to changes in temperature, BOD_5, total phosphorus and suspended solids. Winter and Duthie (2000a) showed that the differentiation of periphytic diatom assemblages along an urban-rural gradient was related to differences in the concentrations of total phosphorus and nitrogen. Ndiritu et al. (2006) found differences in the assemblages of periphytic diatoms in areas with smaller towns and subsistence-farming activities when compared to assemblages found in large urban centers and areas with intensive agriculture, which was associated with changes in the composition of species and was related to the intensity of pollution. In this study, the difference in periphytic diatom assemblages between urban and rural streams were related to the increased conductivity and total nitrogen values recorded for the urban stream, the higher dissolved oxygen values, COD, BOD_5 and flow in the rural stream (Procrustes - $m^2 = 0.7607$ and $p = 0.0001$). In urban landscapes, impervious surface areas intensify the surface runoff, which carries nutrients (WALSH et al., 2005; PAUL; MEYER, 2008) and contaminants to the streams. Deficiencies in waste treatment systems, in addition to illicit discharges of effluents into urban streams, cause an increase in the concentration of nutrients, especially nitrogen (PAUL; MEYER, 2008). High nitrogen concentrations contribute to the excessive development of algae (PORTER et al., 2008), which may lead to a reduction in the concentration of dissolved oxygen (KANNEL et al., 2007). These conditions were observed in the urban stream, which presented severe conditions for the maintenance of species sensitive to increased concentrations of nitrogen and conductivity.

Increased conductivity values were also considered to be one of the main effects of urbanization to streams (SONNEMAN et al., 2001; WALKER; PAN, 2006), and this result can explain great part of the variability between periphytic diatom assemblages (POTAPOVA; CHARLES, 2003). The influence of this variable on periphytic diatoms has been previously reported in several studies (SONNEMAN et al., 2001; SOININEN et al., 2004; WALKER; PAN, 2006; WINEMILLER et al., 2008).

Factors that are frequently indicated as important for the distribution and abundance of periphytic algae, such as phosphorous (WINTER; DUTHIE, 2000a), pH (PAN et al., 1996) and temperature (DENICOLA, 1996), had no influence on the separation of periphytic diatom assemblages between the streams studied; however, they did contribute to the temporal distribution. As observed in PCA, the temporal variation of these abiotic factors did not show a similar pattern between the urban and rural streams because they reflected the activities developed in their micro-basins, and these micro-basins do not show differences in soil or climate. Changes in the structure of periphytic diatom assemblages were expected in response to seasonal changes in abiotic variables (STEVENSON; PAN, 1999). Nevertheless, changes in physical and chemical variables caused by human activities in the micro-basins of the studied streams were more important for the structuring of the assemblages of the periphytic diatoms than the seasonal variation of abiotic factors. Although both agriculture and urbanization affect rivers and streams, pollution from urban areas is more intense than the pollution detected in rural areas (KANNEL et al., 2007).

Species that had a greater indicator potential for the urban stream have already been identified by other authors as tolerant to nutrient enrichment, such as *Fallacia monoculata* (POTAPOVA; CHARLES, 2007) and *Mayamaea atomus* var. *permitis* (FORE; GRAFE, 2002). Species that are frequently associated with conditions of organic pollution were also indicators for the urban stream, such as *Achnanthes lanceolata* (VAN DAM et al., 1994), *Amphora montana* (LOBO et al., 2002; VAN DAM et al., 1994), *Cyclotella meneghiniana* (LOBO et al., 2002; POTAPOVA; CHARLES, 2007), *Eolimna minima* (RIMET, 2009), *Navicula tenelloides* (VAN DAM et al., 1994; SOININEN et al., 2004), *Nitzschia* cf. *inconspicua* (VAN DAM et al., 1994), *Pinnularia gibba* (LOBO et al., 2002) and *Sellaphora seminulum* (LOBO et al., 2002; SALOMONI et al., 2006; RIMET, 2009). *Caloneis bacillum* was reported by Van Dam et al. (1994) as being typical of mesotrophic environments, and this species was an indicator for the urban environment in the streams studied. *Gomphonema lagenula* was an indicator for the urban stream in this study and was an indicator of nutritionally poor environments in a study by Chessman et al. (2006), and our results suggest a greater range of tolerance to nutrient concentrations for this species. *Achnanthidium minutissimum* tolerates conditions that range from oligotrophic to eutrophic (VAN DAM et al., 1994; POTAPOVA; CHARLES, 2007). Here, this species was recorded as an indicator of the urban stream, which was more affected when compared to the rural stream.

Among the indicator species for the rural stream, there are taxa that have already been observed to be tolerant to conditions that vary from oligotrophic to eutrophic environments, such as *Achnanthes exigua*, *Hantzschia amphioxys, Navicula cryptotenella* (VAN DAM et al., 1994), *Ulnaria ulna* (VAN DAM et al., 1994; POTAPOVA; CHARLES, 2007) and *Achnanthes rupestoides*, which was an indicator of oligotrophic environments in the study of Van Dam et al. (1994) and of meso-eutrophic environments in Hermany et al. (2006). *Amphipleura lindheimeri* was recorded by Lobo et al. (2002) as an indicator of heavily polluted environments, and Lobo et al. (2004b) showed that this species has an intermediate tolerance to pollution. *Sellaphora pupula* preferred less eutrophic environments in the study of Hermany et al. (2006) and polluted to very polluted rivers in Lobo et al. (2002) and in Salomoni et al. (2006). There are other taxa mentioned in the literature that are characteristic of eutrophic environments, for example, *Amphora copulata*, *Tryblionella levidensis* (VAN DAM et al., 1994) and *Frustulia crassinervea* (SALOMONI et al., 2006), which were indicators of the rural stream in this study. *Frustulia vulgaris* was determined to be sensitive to high concentrations of nutrients (KELLY et al., 1998), corroborating the results for the two streams studied herein. Meanwhile, species that showed preference for less severe conditions of this type of pollution were *Fallacia ecuadoriana*, *Fallacia insociabilis*, *Geissleria neosubtropica*, *Luticola dapalis*, *Navicula lohmannii*, *Pinnularia* sp., *Placoneis constans* var. *symmetrica*, *Placoneis disparilis*, *Placoneis hambergii*, *Nupela praecipua*, *Sellaphora* sp. 1, *Sellaphora* sp. 2, *Stauroneis* cf. *kriegeri* and *Stenopterobia schweickerdtii*. No ecological information was found with regard to these species, which suggests that they may be indicators of less affected environments.

Conclusion

There was a clear effect of land-use on abiotic variables of the streams, which was in turn reflected by diatoms. The spatial variation of these assemblages was greater than the temporal variation, and the human influence was more pronounced in urban stream, which showed a reduced richness and increased density due to high values of total nitrogen and conductivity. These conditions may have contributed to the highest number of tolerant species in the urban environment, suggesting that the land-use had a greater influence on the assemblage structure of periphytic diatoms than the seasonal variation of abiotic factors.

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