Acta Limnologica Brasiliensia



Original Article Acta Limnologica Brasiliensia, 2017, vol. 29, e1 http://dx.doi.org/10.1590/S2179-975X1816 ISSN 0102-6712

Seasonal and spatial variation of the epilithic diatoms: case study of an organic pollution gradient in a subtropical region of southern Brazil

Variação sazonal e espacial das diatomáceas epilíticas: estudo de caso evidenciando um gradiente de poluição orgânica em região subtropical do sul do Brasil

Saionara Eliane Salomoni^{1*}, Odete Rocha² and Lezilda Carvalho Torgan³

¹Universidade Estadual do Rio Grande do Sul – UERGS, Av. Tancredo Neves, 210, CEP 97670-000, São Borja, RS, Brazil

²Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos – UFSCar, Rodovia Washington Luiz, Km 235, CP 676, CEP 13565-905, São Carlos, SP, Brazil

³Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Rua Dr. Salvador França, 1427, CEP 90690-000, Porto Alegre, RS, Brazil *e-mail: saiosalomoni@hotmail.com

*e-mail: saiosalomoni@hotmail.com

Cite as: Salomoni, S.E., Rocha, O. and Torgan, L.C. Seasonal and spatial variation of the epilithic diatoms: case study of an organic pollution gradient in a subtropical region of southern Brazil. *Acta Limnologica Brasiliensia*, 2017, vol. 29, e1.

Abstract: Aim: This study aimed to understand the seasonal variation of epilithic diatoms in the Gravataí River regarding their composition and density along the river, as well as the respective relationships with local limnological variables. Methods: The diatoms were sampled quarterly using a EDS sampler exposed for four weeks from September 2000 to August 2002. An area of 75 cm² was scraped off the upper surface of three submerged stones totalizing a composed sample. An aliquot of 1 ml of oxidized material was mounted on a slide for the quantitative and qualitative analysis. Results: A spacial organic contamination gradient was observed in the Gravataí river due the decrease of dissolved oxygen, and increase of BOD5 total nitrogen and ortho-phosphate from the upper to the lower course and the predominance of eutrophic conditions during periods of higher temperature (spring and summer). The epilithic diatoms presented, in the upper stretch, a seasonal pattern in abundance with the highest densities during the fall and winter, the density enhance was associated to mild and low temperatures, lowest turbidity and nutrients availability. Bacillariaceae was dominant in the spring, Eunotiaceae and Bacillariaceae in the summer and fall and Gomphonemathaceae was strongly dominant followed by Eunotiaceae in winter. In the lower course Bacillariaceae was dominant in all seasons except on fall 2001. These changes were determined by the substitutions in the occurrence and relative abundance of some species as Achnanthidium minutissimum, Cocconeis placentula, Eunotia bilunaris, Frustulia saxonica, Gomphonema parvulum, Nitzchia palea and Sellaphora seminulum. Conclusions: The seasonality of the diatoms was well evidenced in upper stretch of the river, therefore in the lower course with greater anthropogenic impact, fewer changes occurs in the number of species present and/or of substitution of some species by others, showing that the seasonality of the diatoms can be affected by organic contamination and eutrophication.

Keywords: diatoms; taxonomic composition; density; impacted system.

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Resumo: Objetivo: Este estudo objetivou conhecer a variação sazonal das diatomáceas epilíticas ao logo do Rio Gravataí em relação a composição e densidade, assim como a respectiva relação com variáveis limnológicas locais. Métodos: As diatomáceas foram amostradas trimestralmente utilizando o amostrador EDS exposto por quatro semanas, no período de setembro de 2000 a agosto de 2002. A área de 75 cm² de material foi raspado de três rochas submersas totalizando uma amostra composta. Alíquota de 1 ml de material oxidado foi montado em lâminas para análise qualitativa e quantitativa. Resultados: Um gradiente espacial de contaminação orgânica foi observada no rio Gravataí devido à diminuição do oxigênio dissolvido e aumento do DBO5 nitrogênio total e orto-fosfato do curso superior para o inferior, e a predominância de condições eutróficas durante períodos de maior temperatura (primavera e verão). As diatomáceas epilíticas exibiram em seu curso superior um padrão sazonal em abundância, as mais altas densidades foram registradas no outono e inverno, o aumento da densidade esteve associada as médias e baixas temperaturas, mais baixa turbidez e disponibilidade de nutrientes. Bacillariacea foi dominante na primavera, Eunotiaceae e Bacillariaceae no verão e outono e Gomphonemataceae foi fortemente dominante seguido de Eunotiaceae no inverno. No curso inferior Bacillariaceae foi dominante em todas as estações do ano exceto no outono de 2001. Essas mudanças foram determinadas pela substituição na ocorrência e relativa abundância de algumas espécies como Achnanthidium minutissimum, Cocconeis placentula, Eunotia bilunaris, Frustulia saxonica, Gomphonema parvulum, Nitzchia palea e Sellaphora seminulum. Conclusões: A sazonalidade das diatomáceas foi bem evidenciada no trecho superior do rio, entretanto no curso inferior com maior impacto antrópico, poucas mudanças ocorrem no número de espécies e/ou substituição de algumas espécies por outras, mostrando que a sazonalidade das diatomáceas pode ser afetada pela contaminação orgânica e eutrofização.

Palavras-chave: diatomáceas; composição taxonômica; densidade; sistema impactado.

1. Introduction

Due to their short life cycle, diatoms respond quickly to a variety of environmental conditions, such as organic pollution (Lange-Bertalot, 1979; Lobo, 2013), pH (Pan et al., 1996) and eutrophication (Kelly & Whitton, 1995; Potapova & Charles, 2007; Lobo, 2013). These conditions lead the communities to adjust themselves, modifying their composition through alterations in the number of species and substitution of some of them by others, according to the tolerance limits of each specific environmental condition. The community might also shift due to seasonal cycles, especially in temperate and subtropical regions.

It is well-known that in temperate regions of the Northern Hemisphere, periphytic diatoms are especially abundant during spring in response to increased temperature, light intensity and nutrient availability (Wetzel, 1983; Sherwood et al., 2000). In many lakes, a second peak in abundance of diatoms occurs in the fall (Hoek et al., 1995). According to Sandgren (1988), diatoms in the plankton increase in spring and fall since these are periods of high mixing in the water column.

In a coastal lagoon from the subtropical region of southern Brazil, the seasonal variations in the diatom biomass were different along hyaline zones. In the limnetic zone, maximum biomass was recorded in autumn, the end of winter and spring; in the oligo-mesohaline zone, the maxima were in autumn and spring; and in the oligo-polyhaline zone, the maximum biomass was in summer. The seasonality of the planktonic diatoms in the lagoon was associated with the inflow-outflow of continental and coastal waters and with the silicon concentration (Torgan et al., 1998).

Seasonal variations of diatoms in lotic environments have not been reported for the subtropical region. In general, prior studies have dealt with the composition, structure and substitution of the periphytic community in relation to organic pollution and eutrophication (Oliveira & Schwarzbold, 1998; Rodrigues & Lobo, 2000; Wetzel et al., 2002; Lobo et al., 2003). Specifically for the Gravataí River, prior studies have focused on limnological characterizations, assessing the magnitude of pollution sources (Leite et al., 1996; Salomoni et al., 2007b), and on the water quality based on epilithic diatoms (Salomoni et al., 2006, 2011).

This study aimed to distinguish the seasonal variation of epilithic diatoms in the Gravataí River regarding their composition and density along the upper, medium and lower courses of the river, as well as the respective relationships with local limnological variables.

2. Material and Methods

2.1. Study area

The Gravataí River basin is located in the northeastern region of the State of Rio Grande do Sul $(29^{\circ}45' - 30^{\circ}12'N ; 50^{\circ}27' - 51^{\circ}12'W)$

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Figure 1. Location of the Gravataí River watershed, in the state of Rio Grande do Sul, and of the six sampling stations along the Gravataí River. Stations: 1 = Chicolomá stream, city of Santo Antônio da Patrulha; 2 = Juca Barcelos farm, city of Glorinha; 3 = Passo dos Negros, city of Gravataí; 4 = Pumping station, city of Porto Alegre/Canoas; 5 = Areia Stream, city of Porto Alegre/Canoas; 6 = Gravataí River mouth, city of Porto Alegre.

(Figure 1). Its springs are formed by steep slopes, at altitudes of up to 400 m, and its water course reach and pouring them in Banhado Grande, that consequently act as a regulator of the river flow. The relatively mild topography in the middle and lower course does not favor the formation of microclimatic abnormalities of considerable magnitude (IPH, 2002). This basin has two regions of distinct soil usage: the upper part of the river with intense agricultural activity and the lower part with major urban and industrial uses (Leite et al., 1996).

2.2. Sampling and analysis

Diatoms were sampled quarterly at six stations along the river (Figure 1) using Epilithic Diatom Samplers (Salomoni et al., 2007a), from September 2000 to August 2002. The exposure time of rocks in the river was about four weeks, according to the procedures recommended by Lobo & Buselato-Toniolli (1985). In each station, an area of 75 cm² of material was scraped off the upper surface of three submerged stones using a toothbrush, totalizing a composed sample preserved with formaldehyde 4%. The material was oxidized following the procedures described by Kobayasi & Mayama (1982), and an aliquot (1 ml) was mounted with the Naphrax[®] resin on a permanent slide for quantitative and qualitative analyss. For each station, at least 400 valves were counted, according to the methodology established by Schoeman (1973). The results were expressed in number of ind./cm².

For species identification, the following references were used: Patrick & Reimer (1966, 1975),

Krammer & Lange-Bertalot (1986, 1988, 1991a, b), Metzeltin & Lange-Bertalot (1998, 2002), Krammer (2000), Rumrich et al. (2000) Lange-Bertalot (2001), Metzeltin et al. (2005) and Bes et al. (2012). The Lobo & Leighton (1986) criteria were followed to determine abundant and dominant species. The samples were deposited in the Herbarium Prof. Dr. Alarich R. H. Schultz (HAS 103699-103813), located in the Museum of Natural Sciences, Fundação Zoobotânica of Rio Grande do Sul.

Measurements of temperature (TEMP), conductivity (COND), turbidity (TURB), pH, and concentration of dissolved oxygen (DO) were carried out at each sampling. In the laboratory, analyses of biochemical oxygen demand after 5 days (BOD5), chemical oxygen demand (COD), ammonium concentrations (AMN), total nitrogen (TN), organic nitrogen (ON), dissolved orthophosphate (OP), total phosphate (TP), chloride (CHLO) and thermotolerant coliforms (TC) were performed according to APHA (2005). These data were provided by the State Environmental Foundation and the Municipal Department of Water and Sewage in Porto Alegre.

2.3. Data processing

Using a matrix of relative abundance of species vs. samples, the indicator species were assembled by TWINSPAN, as described by Dufrêne & Legendre (1997) resulting in three groups of species. The Canonical Correspondence Analysis (CCA) was used to reveal the main gradients of the variation in species composition corresponding to the degree of organic pollution and eutrophication. The analyses were performed with PC-ORD version 4.0 of MjM Software Design for Windows (McCune & Mefford, 1999). The first canonical axes were tested for significance by the Monte Carlo Test (999 permutations) at the 5% level. A correspondence analysis was also performed to relate the occurrences of abundant species with the seasons of the year. Pearson correlation analysis (p<0.05) was carried out between the diatom density and abiotic data. Correlation analyses were performed using the Statistica® software.

3. Results

3.1. Environmental variables

Rain was distributed evenly throughout the sampling period (Figure 2). The annual precipitation between 2000 to 2002 were 1323 mm in 2000, 1536 mm in 2001, and 1496 mm in

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2002. Winter precipitation was low, with a mean of 88.5 mm in 2001. The highest value was recorded in January 2001 (total of 298.9 mm) and lowest (25 mm) in August 2001. The monthly maximum and minimum temperatures (°C) are shown in Figure 3. Variations in temperature followed the typical pattern of warm summers with mean air temperatures around 30°C and cold winters around 20°C. The fall and spring had more mild temperatures with mean values between 22 and 25°C (Figure 3).

The Gravataí River showed during the period of study low flow (<5 m³/s), only in the middle course, during fall, high flow rates were observed reaching a maximum value of 39.7 m³/s in May 2001. Regarding limnological variables (Table 1), we found a decrease in the concentration of dissolved oxygen and turbidity, and an increase of BOD₅, total nitrogen and ortho-phosphate from station 1 to 6. A rising number of thermotolerant coliforms were also observed along the river. These results reveala pollution gradient from the upper to lower course.

3.2. Community and environmental variables

The canonical correspondence analysis (CCA) of the community and environmental variables (Figure 4) explained 30.2% of the total variability



Figure 2. Monthly precipitation variation (mm) during the period of January 2000 to October 2002. Source: Rio Grande do Sul Agricultural Research Institute Station located in Gravataí River Basin.



Figure 3. Monthly maximum and minimum temperatures (°C) from January 2000 to October 2002. Source: Rio Grande do Sul Agricultural Research Institute Station.

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Table 1. Mean (n=8) and standard deviation (SD), of limnological variables from September 2000 to August 2002, at Chico Lomá stream (station 1) and Gravataí River (stations 2 to 6). Data not measured (---) Data.

Stations	1		2		3		4		5		6	
Variables	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
TEMP (°C)	22.5	4.92	20.81	4.22	2 23.00	5 5.05	22.34	4.87	22.75	4.95	21.76	5.01
TURB (TNU)	72.70	43.60	55.57	31.83	7 71.58	3 30.33	66.26	20.79	67.06	26.53	37.76	16.28
COND (µS cm⁻¹)	73.12	36.44	52.84	17.03	4 49.81	1 16.20	104.09	45.45	124.98	56.21	139.09	66.21
CHLO (mg L ⁻¹ CI)	8.04	4.07	5.85	1.68	7 7.13	2.71	11.25	5.28	11.91	5.70	13.38	4.51
pН	6.22	1.36	6.30	0.35	6.37	0 0.41	6.40	0.45	6.45	0.43	7.02	0.27
DO (mg L ⁻¹ O ₂)	5.65	2.13	4.74	1.77	4.70	2 2.01	2.50	1.53	2.00	1.23	3.45	2.32
BOD (mg L ⁻¹ O ₂)	1.45	0.80	1.20	0.52	1.36	0. 0.95	3.64	1.71	5.43	3.23	6.81	4.38
COD (mg L ⁻¹ O ₂)	35.70	13.13	32.28	9.95	3 38.74	9. 9.78	40.87	10.63	42.39	11.34	38.84	9.16
AMM. (mg L ⁻¹)	0.30	0.14	0.15	0.07	0.36	0. 0.53	2.16	1.99	2.87	2.78	3.37	2.70
ON (mg L ⁻¹)	1.08	0.65	0.65	0.19	0.89	0. 0.35	1.49	1.06	2.40	2.53	1.78	1.01
TN (mg L ⁻¹)	1.12	0.75	0.77	0.26	1.27	0. 0.84	3.82	2.77	4.97	4.64	5.27	3.01
OP (mg L ⁻¹)	0.06	0.04	0.03	0.02	0.05	0.0.08	0.26	0.21	0.30	0.25	0.82	0.61
TP (mg L ⁻¹)	0.21	0.19	0.17	0.23	0.16	0. 0.14	0.51	0.35	0.62	0.41	1.32	1.14
TC (MPN 100 ml ⁻¹)	313	248	271	207	859	1 10001	49304	59381	95175	99623	175120	186048



Figure 4. CCA biplot showing the ordination of 40 sampling sites based on TWINSPAN groups of diatom species composition (Salomoni et al., 2006). **CL** = chlorides; **COLI** = thermotolerant coliforms; **COND** = conductivity; **BOD**₅ = biochemical oxygen demand after five days; **COD** = chemical oxygen demand; **NH**₃ ammonium; **TN** = total nitrogen; **DO** = dissolved oxygen; **PO**₄**O** = orthophosphate. Dashed arrow indicates pollution gradient.

of data in its first two axes, which showed eigenvalues of 0.414 and 0.308, respectively. The species/environment correlation coefficients obtained for axes 1 (r = 0.913) and 2 (r = 0.837) indicated a strong correlation between the distribution of species and the environmental variables used in the ordination. The Monte Carlo permutation test revealed that the ordination of these axes was statistically significant (p < 0.01). These results revealed that axis 1 explained 17.3% of the variability highlighting the chloride (0.417), associated with its positive side. In the intra-set correlations, conductivity, chloride, BOD₅, COD, ammonium, total nitrogen, orthophosphate, and thermotolerant coliforms were highlighted as variables positively correlated to axis 1, showing a clear gradient of organic contamination and eutrophication. Axis 2 (12.9% of explained

variation), also presented a high correlation with BOD_5 , associated with the negative end of the axis, where the intra-set correlation ratified this variable as the most significant (r = -0.594). It also highlights the predominance of eutrophic conditions during periods of higher temperature (spring and summer) as opposed to periods of lower temperature (winter and fall).

Data of the upper course (stations 1, 2 3) and lower course (stations 5, 6 7) were analyzed separately, based on the cluster analysis of the physical, chemical and microbiological conditions (Salomoni et al., 2007b).

Figure 5 shows the seasonal variation of diatom density and the selected environmental variables. The seasonal variations in density were observed in the upper stretch of the river (stations 1-3).



Figure 5. Seasonal variation of the mean values of total diatom density (ind cm²) and of the environmental variables (Water temperature (°C), precipitation (mm), orthophosphate (mg L⁻¹), total phosphorus (mg L⁻¹), total nitrogen (mgN L⁻¹) and turbidity (NTU), during different seasons, sampled in the period from September 2000 to August 2002 (Sp = spring; Su = summer; F = fall; W = winter).

In general, highest densities were observed during the fall and winter (Figure 5).

Water temperature (Figure 5A) negatively correlated (r = -0.895) with the density in the summer of 2001 in the upper course of the river as well as in the lower course, the period in which the highest precipitation was recorded with 298.9 mm (Figure 5B). However, during the fall of 2002, the correlation was positive (r = 0.842) in the upper course of the river.

The highest value of orthophosphate was recorded in the upper stretch (stations 1, 2 and 3) in the summer, and in the lower stretch of the river (stations 4, 5 and 6) in the fall of 2001 (Figure 5C). These values did not correlate with diatom density. Total phosphorus (mg. L⁻¹) negatively correlated (r = -0.921) with diatom density in the lower course of the river in the winter (Figure 5D). Total nitrogen negatively correlated (r = -0.960) with the density of the algae in the upper course of the river in the winter (Figure 5E) and the correlation of the diatom density with the turbidity was negative (r = -0.921) in

both the upper and lower stretches of the Gravataí River (Figure 5F).

3.3. Community composition

The epilithic diatoms of the Gravataí River were composed by a total of 169 taxa in specific and infra-specific levels, distributed in 43 genera and 25 families. The main families based on the relative species number were Eunotiaceae (13.6%), Naviculaceae (11.24%); Bacillariaceae (10%); Pinnulariaceae (9.46%); Gomphonemathaceae (6.50%); Fragilariaceae and Achnanthaceae (5.32%).

A more clear seasonal variation was observed in the relative abundance of the main taxonomic families in the upper course (stations 1) when compared to the lower course of the river (station 6) (Figure 6). At station 1, Bacillariaceae was dominant during spring, in summer 2001 Eunotiacae and Bacillariaceae were the most abundant families, and both families remained well represented in the fall, there was the dominance of



Figure 6. Temporal variation of relative abundance of the main diatom families at station 1 and station 6, correspondent to the upper and lower stretches of the Gravataí river, RS, sampled during the period of September 2000 to August 2002. (Sp = spring; Su = summer; F = fall; W = winter). The sample Sp1/02 was lost.

Sellaphoraceae, in both years investigated. In the winter, the Gomphonemathaceae family was strongly dominant followed by Eunotiaceae in 2002. At station 6 there we not observed a distinct pattern of seasonality, Bacillariaceae was dominant in all seasons of the year (exception on the fall 2001) when there was dominance of the Sellaphoraceae. The changes that occurred in diatom community composition were determined by the substitutions in the occurrence and in the relative abundance of some species (Figure 7).

At station 1, *Nitzchia palea* was the dominant species in both summer periods, while *Sellaphora seminulum* was dominant in fall. In the winter, *Gomphonema parvulum* was dominant then substituted by *Eunotia bilunaris* in both seasonal cycles. At stations 2 and 3, this same pattern was observed, *Nitzschia palea* being dominant in the spring at station 3, *Sellaphora seminulum* in the fall and *Gomphonema parvulum* in the winter, in both stations. At stations 4, 5 and 6, *Nitzschia palea* was dominant during all seasons, being substituted by *Sellaphora seminulum* in some occasions just before spring.

The variation in density of some species is shown in Figure 7. In both seasonal cycles, the species that were most abundant were *Gomphonema parvulum*, *Nitzschia palea*, *Selllaphora seminulum* and *Achnanthidium minutissimum*. While some species were present in all seasons, such as *Nitzchia palea*, *Gomphonema parvulum* and *Sellaphora seminulum*, others only occurred in one season, such as *Cocconeis placentula*, which was restricted to colder periods of fall and winter.

4. Discussion

In the upper stretch of the Gravataí River, the epilithic diatoms had a seasonal pattern in abundance, the highest density was recorded in the fall and winter, fall for both evaluated seasonal



Figure 7. Variation of mean values and standard deviations in the density of the most abundant diatoms (ind/cm²), in the upper (stations 1,2,3) and lower (stations 4,5,6) stretches of the Gravataí river, from September 2000 to August 2002 (Sp=spring; Su=summer; F=fall; W=winter).

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cycles, the density enhance during these seasons were associated to mild and low temperatures, lowest turbidity and nutrients availability.

According to Patrick (1977), the most important density-independent factors that affect diatom communities are light, temperature, and flow rate of the water; while the nutrient levels have more effect on diatom species. Our study on the Gravataí River was able to prove this statement.

In the subtropical zone, light is available throughout the year, so it is not a limiting factor. The temperature effects diatom growth and certain species have a narrow temperature range and others are able to withstand rather large ranges so the range between 15 and 25°C during the fall and winter was more propition to species from the upper stretch of the river.

Turbidity can decrease light penetration affecting diatom growth (Patrick, 1977). The lowest values of turbidity between 40.00 and 50.00 NTU were observed during the fall and winter and were capable to permit light absorption by the diatoms.

Fall and winter were also the periods of higher flow in the middle stretch of the Gravataí River due to high precipitation in May, June and July, which increased turbulence in the water column and for the epilithic diatoms attached to the substratum. The Gravataí river is situated in a plain region and its flow rate is low, thus increasing the flow and turbulence in the system may mean an early renewal of nutrients around the cells, favoring higher nutrient availability. Within a specific range of water turbulence and current velocity, the boundary layer thickness between the periphyton and overlying water is reduced and enhanced nutrient exchange occurs as a result (Riber & Wetzel, 1987; Wetzel, 2005).

In the upper stretch of the Gravataí River, a higher concentrated oxygen and lower concentration of nutrients were observed. In the lower stretch, the higher increase in labile organic matter, derived from wastewater dumped in natura, reflected in the values of BOD and of fecal coliforms, which increased on average 3 to 50 times. At station 6, near the spring, the nutrient concentrations and density of coliforms were even higher, around 10 and 100 times, respectively. In general, the river in its lower stretch reduce its ability of re-aeration and of dilution of pollutants and its self-purification is limited for now having an insufficient dilution, small surface contact and absence of turbulence (Porto Alegre, 1972, 1981; IPH, 2002). The environmental gradient was evident in the river due the decrease

of dissolved oxygen, and increase of BOD_{5} , total nitrogen and ortho-phosphate from the upper to the lower stretch, affecting the seasonal variation of the community composition.

In the upper stretch of the river, the epilithic diatoms showed seasonal changes in densities and species composition. *Achnanthidium minutissimum*, *Cocconeis placentula*, *Frustulia saxonica* and *Sellaphora seminulum* were species typically abundant during fall, while *Gomphonema parvulum* reached higher densities in the fall and winter.

In the lower stretch of the river, the epilithic diatoms did not show the same seasonal pattern. Considering that this stretch is eutrophic, it becomes a quite selective environment, favoring only the most tolerant species, such as *Nitzschia palea* and *Sellaphora seminulum*. These species became gradually dominant and permanent along the annual cycle and seasonality was not observed.

We presume that the organic contamination gradient and eutrophication of the rivers affect the seasonal community pattern, based on investigations carried out by Rodrigues et al. (2007) on the phytoplankton in the rivers of the Guaiba Basin. This study showed that at Cai, Jacuí, Sinos and Gravataí river mouths, Chlorophyceae presented higher species numbers in the summer, while the diatoms were richer in the fall and winter in these rives exception Gravataí River.

Considering another lotic system Sampario stream, situated in the Jacuí Basin,that does not show nitid polution gradient, we can verified also a seasonal pattern. The studies carried out by Oliveira & Schwarzbold (1998) and Rodrigues & Lobo (2000) revealed the substitution of *Cocconeis placentula*, abundant during the fall and spring, by species of the genera *Navicula, Gomphonema* and *Synedra* during other periods of the year In the same stream, higher densities of *Achnanthidium minutissimum, Gomphonema parvulum* and *Sellaphora seminulum* were found during the lower temperatures of winter, results that are similar to the ones observed for Gravataí River.

Lobo et al. (2003), evaluating the water quality of the other streams from the same watershed, recorded that *Nitzschia palea* was abundant in all samples, due to organic contamination and eutrophication. In the same system, *Sellaphora seminulum*, *Geissleria aikenensis*, *Nitzschia palea*, *Gomphonema parvulum* and *Navicula rostellata* were also most abundant during fall.

The species composition is an attribute able to give us valuable information on the environmental

conditions and seasonality. This case study revealed that in the environment with greater anthropogenic impact, fewer changes occurs in the number of species present and/or of substitution of some species by others, according to their limits of tolerance, showing that the seasonality of the diatoms can be affected by pollution and eutrophication.

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Received: 18 March 2016 Accepted: 02 December 2016