

Limnological characterisation and phytoplankton seasonal variation in a subtropical shallow lake (Guaíba Lake, Brazil): a long-term study

Caracterização limnológica e variação sazonal do fitoplâncton em um lago raso subtropical (Lago Guaíba, Brasil): estudo de longa duração

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Abstract: Aim: to provide a long-term limnological characterisation of a subtropical shallow lake in addition to verifying seasonal differences, including phytoplankton variation. **Methods:** monthly sampling at sites IP, SJ and MD from 2000 to 2009 to analyse temperature - T; depth - Z; the depth of the euphotic zone - Z_{eu} ; Z_{eu}/Z (%); total suspended solids – TSS; dissolved oxygen – DO; pH; electrical conductivity - EC; N-NH₃, N-NO₂, N-NO₃; soluble reactive phosphorus - SRP; chlorophyll a - Chl-a and phytoplankton. **Results:** low values of Z and Z_{eu} characterised the shallow and turbid conditions of lake and corresponded to the contribution of nano-microflagellates (*Chlamydomonas sp.*, *Spermatozopsis sp.*, *Cryptomonas sp.* and *Rhodomonas sp.*) and diatoms (*Aulacoseira granulata*). Z_{eu}/Z (%), SRP and Chl-a were significantly different at site IP (meso-eutrophic) compared to sites SJ and MD (eutrophic). Phytoplankton density was also significantly higher at sites SJ and MD, and the largest relative contribution of *Actinastrum sp.*, *Dictyosphaerium sp.*, *Micractinium sp.*, *Monoraphidium sp.*, *Scenedesmus/Desmodesmus sp.* and *Euglena sp.* corresponded to the most polluted waters at site SJ. The significantly higher T (°C) in summer corresponded to significantly higher Chl-a as well as a greater richness and density of phytoplankton. *Cocconeis sp.*, *Gomphonema sp.* and *Pinnularia sp.* (pennated diatoms) were negatively correlated with temperature and were therefore more representative at the three sites in winter. *Asterionella formosa* was correlated with SRP and vernal blooms were recorded (2000-2001). *Planktothrix isoethrix* and *Planktothricoides raciborskii* were expressive in the summer/late summer (2004-2005), and were significantly correlated with Chl-a and low SRP in water column. **Conclusions:** The study corroborated the sensitivity of phytoplankton in characterising different stages of eutrophication at different sites and corresponding watersheds as well as in characterising different seasons in a shallow lake in the subtropical zone of Brazil.

Keywords: freshwater, algae, cyanobacteria, eutrofication.

Resumo: Objetivo: prover caracterização limnológica de longa duração e verificar as variações sazonais associadas ao fitoplâncton em um lago raso subtropical no sul do Brasil. **Métodos:** coletas mensais em três locais (IP, SJ e MD), de 2000 a 2009, para as seguintes análises: temperatura - T; profundidade - Z; profundidade da zona eufótica - Z_{eu}/Z (%); sólidos suspensos – TSS; oxigênio dissolvido – DO; pH; condutividade - EC; N-NH₃, N-NO₂, N-NO₃; fósforo solúvel - SRP; clorofila-a - Chl-a e fitoplâncton. **Resultados:** baixos valores de Z e Z_{eu} caracterizaram a condição turbida e rasa do lago e corresponderam à contribuição de *Aulacoseira granulata* e de flagelados nano-microplancctônicos dos gêneros *Chlamydomonas sp.*, *Spermatozopsis sp.*, *Cryptomonas sp.* e *Rhodomonas sp.* dos grupos X₂ e Y. Z_{eu}/Z (%), SRP e Chl-a foram significativamente menores no ponto IP (meso-eutrófico) comparado aos pontos SJ e MD (eutróficos) onde a densidade do fitoplâncton foi significativamente maior. A maior contribuição relativa de gêneros *Actinastrum sp.*, *Dictyosphaerium sp.*, *Micractinium sp.*, *Monoraphidium sp.*, *Scenedesmus/Desmodesmus sp.* e *Euglena sp.* ocorreu nas águas mais poluídas do ponto SJ. T média significativamente maior no verão correspondeu aos maiores valores médios de Chl-a e às maiores riqueza e densidade do fitoplâncton. Diatomáceas penadas (*Cocconeis*

sp., *Gomphonema sp.*, *Pinnularia sp.*) foram negativamente correlacionadas à T e foram mais representativas no ponto IP, provavelmente em função da maior vazão do rio Jacuí. *Asterionella formosa* foi correlacionada ao SRP e florações verais foram registradas entre 2000 e 2001. *Planktothrix isothrix* e *Planktothricoides raciborskii* foram expressivas no verão dos anos de 2004 e 2005 e foram significativamente correlacionadas à Chl-a e associadas à períodos de baixos valores de SRP na coluna d'água. **Conclusões:** o estudo confirmou a sensibilidade do fitoplâncton em caracterizar diferentes estágios de eutrofização, bem como, caracterizar diferentes estações do ano em um lago raso subtropical do Brasil.

Palavras-chave: água doce, algas, cianobactérias, eutrofização.

1. Introduction

The artificial enrichment of inland waters with nutrients, mainly N and P, results from sewage discharge from urban, industrial and agricultural regions (Huszar et al., 2005). This process, referred to as eutrophication, has worldwide negative repercussions, causing serious damage to water supplies, notably during treatment for human consumption (Tundisi et al., 2006; McGowan et al., 2012), and changes in aquatic ecosystems and related biological communities, mainly phytoplankton (Istvánovics, 2009).

Phytoplankton represents an important food source for consumers in the pelagic zone of inland waters and is important when assessing water quality (Demir and Atay, 2002), as it acts as a refined sensor of environmental conditions and an accurate indicator of environmental changes (Alvarez-Cobelas et al., 1998). Phytoplankton communities are generally grouped into classes for taxonomic description and for summarising the ecological status of aquatic environments. More recently, phytoplankton functional groups or assemblages, which are groupings of species and genera based on similar sensitivities and tolerances, have been proposed to improve analyses of ecological status and water quality (Reynolds et al., 2002; Reynolds, 2006; Padišák et al., 2006, 2009).

Because the growth of planktonic algae and cyanobacteria is associated with the availability of light and nutrients (Reynolds, 2006), characterisation of phytoplankton is important in describing trophic states and in limnological characterisation (Bellinger and Sigeo, 2010), as there is a direct relationship between planktonic dynamics and nutritional levels associated with rivers, dams and lakes (Wetzel, 1983).

Phytoplankton varies in time and space at different scales (Abreu et al., 2010). Models have related phytoplankton variability to nutrient concentrations in inland waters of temperate zones

due to the marked seasonality in these regions (Huszar et al., 2005). Several long-term studies have been performed to establish not only patterns of phytoplankton fluctuations but also to verify alterations in these patterns over the years due to hydrological, nutritional and climatic changes (Elliott, 1990; Anneville et al., 2004).

In tropical zones, temperature is not a limiting factor for phytoplanktonic development. Hydrology, the morphometry of water bodies, the use and occupation of watersheds and the frequency, distribution and amount of rain, in addition to the velocity and direction of winds are the most important factors for explaining the occurrence, structure and variation of phytoplankton (Huszar, 1996).

Brazil is a continental country located between the tropical and subtropical zones, and its inland waters are mainly represented by streams, rivers, dams and shallow coastal lakes and lagoons. A study conducted across Brazil showed that the conditions in most of its inland water bodies ranged from mesotrophic to eutrophic (Abe et al., 2006) due to population and economic growth in their corresponding watersheds, which has compromised the quality of water supplies and of water for other uses, mainly due to the occurrence of cyanobacterial blooms (Sant'Anna et al., 2008). Despite its significance for predicting events and the identification of environmental changes, such as those related to climate, hydrology, eutrophication and oligotrophication (Barbosa and Padišák, 2004; Valdes-Weaver et al., 2006), long-term limnological studies in inland waters are scarce in Brazil (Seeliger et al., 2002).

The aim of this study was to provide a long-term limnological characterisation of a subtropical shallow lake from southern Brazil (Guaíba Lake), in addition to verifying seasonal differences, including the influence of qualitative-quantitative phytoplankton variation.

2. Material and Methods

2.1. Study area

Guaíba Lake ($29^{\circ}55' - 30^{\circ}24' S$; $51^{\circ}01' - 51^{\circ}20' W$) is located in the Central Depression of Rio Grande do Sul State, Brazil. It extends from the north, in the region of the mouths of rivers that form the Jacuí Delta, to the south, in the Itapua tip, where it flows into Patos Lagoon (Figure 1). The length of the lake is approximately 50.0 km, and its width ranges from 0.9 to 19.0 km. The surface area of the lake is approximately 496 km² (Nicolodi et al., 2010), and its depth is highly variable, averaging two meters (Bendati et al., 2003).

The rivers that form the Jacuí Delta contribute an average flow of 2,193 m³.s⁻¹, with 84.6% of the volume being attributed to the Jacuí River, 7.5% to the dos Sinos River, 5.2% to the Caí River and 2.7% to the Gravataí River. As carriers of sediment, the rivers lose competence toward the broad depositional basin, and the sedimentation rates of coarse material range from 3.5 to 8.3 mm.year⁻¹. Guaíba Lake stores a volume of approximately 1.5 billion m³ of water. The river output, water level fluctuations in Patos Lagoon and the direction and intensity of winds are the main factors controlling the flow dynamics in this area. Thus, the lake is not only an extension of the rivers but represents a type of tank that is closely linked to Patos Lagoon (Nicolodi et al., 2010).

In the region of the lake, the climate is humid subtropical (soft mesothermal), categorised as Cfa in Köppen classification (Köppen, 1948). The average minimum and maximum air temperature values range from 14.8 °C (winter) to 24.2 °C (summer), and the annual average accumulated precipitation ranges from 1,324 mm (Livi, 1998) to 1,366 mm (Vieira and Rangel, 1988).

Guaíba Lake is an important water supply source for the surrounding cities as well as supporting other uses related to economic and commercial activities (Bendati et al., 2003). The most frequent perturbations in this ecosystem are caused by agriculture processes, urbanisation and domestic and industrial sewage discharges (Terra et al., 2009). Five water intake facilities are operated by the Departamento Municipal de Água e Esgotos – DMAE (water and wastewater public works) of Porto Alegre city, three of which have not changed the location of water intake and have been monitored by DMAE since the 1970s. This database constitutes a potential data source for long-term limnological studies and production of information (Andrade and Giroldo, 2010).

2.2. Sampling and analysis

Sampling was carried out monthly between January 2000 and December 2009. The samples were collected just below surface, in the water column at three sites ($n = 360$): Ilha da Pintada - IP; São João/Navegantes – SJ; and Menino Deus - MD

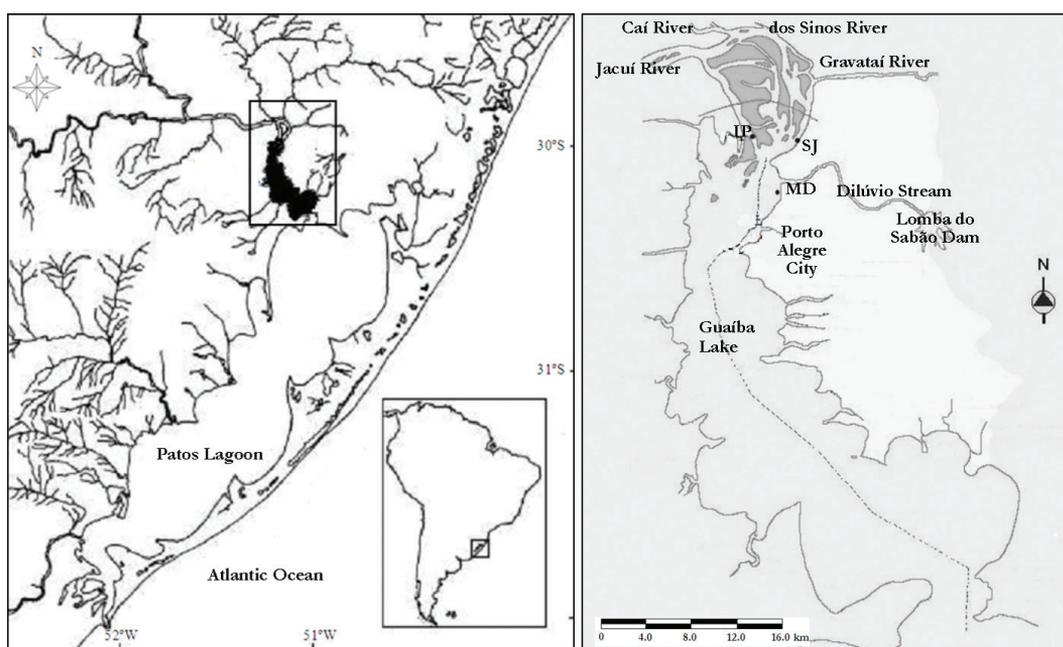


Figure 1. Study area and the IP, SJ and MD sampling sites in Guaíba Lake, Rio Grande do Sul State, Brazil.

(Figure 1). The field analysis included the measurements of the following parameters: water temperature ($^{\circ}\text{C}$) – T, using an alcohol thermometer; depth (m) – Z, using a graduated weighted rope and transparency (m), using a Secchi disk. The depth of the euphotic zone - Z_{eu} (m) was calculated as three times the Secchi disk extinction depth (Cole, 1994). In the laboratory, the following limnological variables were analysed: total suspended matter (mg.L^{-1}) – TSS, via a gravimetric method (ABNT, 1989); dissolved oxygen (mg.L^{-1}) – DO, using the Winkler volumetric method (ABNT, 1988); pH and electrical conductivity ($\mu\text{S.cm}^{-1}$) – EC, with an electrometric method (ABNT, 1999a, 1999b); and ammonia – N-NH_3 , nitrite – N-NO_2 , nitrate – N-NO_3 and soluble reactive phosphorus ($\mu\text{g.L}^{-1}$) – SRP, via ion chromatography (USEPA, 1986, 1993). Chlorophyll-*a* - Chl-*a* ($\mu\text{g.L}^{-1}$) determination was carried out following 90% acetone extraction through spectrophotometric analysis (APHA, 1998).

For phytoplankton analyzes the samples were collected by direct passage of bottles in the sub-surface of the water column. During transport (up to 2 hours), the samples remained chilled (8°C) to its preservation in the laboratory. Non-preserved aliquots were analysed qualitatively under a microscope to survey algae genera, which were identified mainly according Bicudo and Bicudo (1970), Bicudo and Menezes (2005) and Bourrelly (1970, 1972, 1981). Some previously identified species were recorded in this study. Aliquots were preserved with 0.5% acetic Lugol solution (Vollenweider, 1974) Due to the large number of samples collected and analyses performed, several Brazilian sanitation companies adopt quick protocols for phytoplankton identification and quantification in order to standardize results. Tests between laboratories have shown similar and satisfactory results obtained using counting chambers (Sedgwick-Rafter) compared to the traditional sedimentation method with Utermöhl chambers (Müller et al., 2010). Thus phytoplankton was quantified in Sedgwick-Rafter chambers (length: 50 mm; width: 20 mm; height: 1 mm, volume: 1 mL) with the aid of a Whipple reticule coupled to the ocular of a light microscope. The *genera* were quantified in random fields or bands, and whenever possible, one hundred individuals of a predominant genus were quantified as described in APHA (1998).

The genera were grouped into taxonomic classes according to Van den Hoek et al. (1995). The algae

genera that, on average, represented 0.1% or more of the total density of the samples were considered to represent descriptive taxa (Bicudo et al., 2006).

Descriptive and inferential statistics were determined using the *Kruskal-Wallis* (K-W) test at a significance level of at least 95% ($p < 0.05$) for the set of limnological variables and the data on the richness and density of phytoplankton (log transformation), and all data were grouped into sites and seasons. The data were framed by season according the official calendar of the U.S. Naval Observatory (USNO) for the southern hemisphere, where the summer conventionally starts on December 21, autumn on March 21, winter on June 21 and spring on September 23.

To summarise the obtained data, a matrix of the standardised means (z scores) of the limnological variables and phytoplankton richness and density data for each season at each site was used to perform a principal component analysis (PCA). Pearson's correlations were provided to investigate the relationships between the densities of descriptive taxa with limnological variables at $p < 0.05$ significance level. All descriptive, inferential and multivariate statistics were obtained using Paleontological statistics software package for education and data analysis - PAST freeware (Hammer et al., 2001).

3. Results

3.1. Limnological characterisation

The water temperature varied from 10.4°C at the SJ site to 29.5°C at both the SJ and MD sites (Table 1). IP was the shallowest site, although the average Z_{eu} values were not significantly different between the sites. However, the Z_{eu}/Z ratio (%) was significantly higher at IP in relation to the other sites, while that at MD was significantly lower in relation to the SJ site. The MD and SJ sites were significantly different from site IP in terms of higher values of Z, N-NO_2 , SRP and Chl-*a* and lower values of pH and DO. The SJ and MD sites were significantly different in that a higher value of Z was observed at the MD site, while higher values of the Z_{eu}/Z ratio (%), TSS, EC and N-NH_3 were found at the SJ site. On the other hand, the highest concentration of N-NO_3 was detected at the MD site, which was not significantly higher at the IP and SJ sites, while the Chl-*a* values were significantly lower at site IP, by almost two times, in relation to sites SJ and MD.

Table 1. Sites and seasonal mean \pm sd of variables in Guaíba Lake and *K-W* test results (p-values); different letters: significant differences of values at $p < 0.05$.

Variable	Site	p-value (sites)	Summer	Autumn	Winter	Spring	p-value (season)
T (°C)	IP		26.8 ^a \pm 1.4	19.3 ^b \pm 3.5	15.9 ^c \pm 2.0	21.6 ^d \pm 2.5	<0.001
	SJ		27.4 ^a \pm 1.2	19.3 ^b \pm 3.5	15.9 ^c \pm 2.0	21.9 ^d \pm 2.5	<0.001
	MD	>0.05	26.9 ^a \pm 1.5	20.7 ^b \pm 3.2	16.0 ^c \pm 1.6	22.2 ^b \pm 2.5	<0.001
Z (m)	IP		3.6 ^a \pm 0.4	4.0 ^b \pm 0.5	4.2 ^b \pm 0.5	4.1 ^b \pm 0.6	<0.001
	SJ		6.2 ^a \pm 0.5	6.7 ^b \pm 0.5	6.8 ^b \pm 0.5	6.8 ^b \pm 0.9	<0.001
	MD	<0.001	9.5 ^a \pm 0.9	9.8 ^b \pm 0.7	10.1 ^b \pm 1.0	10.0 ^b \pm 1.0	<0.05
Z _{eu} (m)	IP		1.1 ^a \pm 0.5	1.9 ^a \pm 1.7	1.1 ^a \pm 0.5	1.1 ^a \pm 0.5	>0.05
	SJ		1.5 ^a \pm 0.4	1.4 ^{ab} \pm 0.5	1.1 ^{bc} \pm 0.3	1.0 ^c \pm 0.4	<0.001
	MD	>0.05	1.5 ^a \pm 0.4	1.5 ^a \pm 0.6	1.2 ^b \pm 0.4	1.1 ^b \pm 0.4	<0.05
Z _{eu} /Z(%)	IP		43.9 ^a \pm 2.8	50.5 ^a \pm 4.6	25.6 ^b \pm 12.1	28.5 ^b \pm 16.5	<0.05
	SJ		23.5 ^a \pm 7.7	20.3 ^b \pm 8.4	15.7 ^{bc} \pm 5.3	15.4 ^c \pm 14.957	<0.001
	MD	<0.001	15.7 ^a \pm 5.7	15.2 ^a \pm 6.9	11.6 ^b \pm 5.4	11.1 ^b \pm 5.5	<0.05
TSS (mg.L ⁻¹)	IP		12.8 ^a \pm 8.2	19.2 ^{ab} \pm 16.4	26.5 ^b \pm 23.4	26.7 ^b \pm 22.2	<0.05
	SJ		17.9 ^a \pm 7.7	20.4 ^{ab} \pm 10.4	25.4 ^b \pm 10.7	27.6 ^b \pm 17.6	<0.05
	MD	<0.05	16.0 ^a \pm 7.6	17.1 ^{ab} \pm 8.8	24.0 ^b \pm 1.5	23.6 ^b \pm 1.3	<0.05
DO (mg.L ⁻¹)	IP		7.0 ^a \pm 0.6	8.1 ^b \pm 0.9	8.9 ^c \pm 0.4	7.3 ^d \pm 0.7	<0.001
	SJ		5.4 ^a \pm 0.9	6.0 ^b \pm 1.1	6.8 ^c \pm 1.0	5.6 ^{abc} \pm 1.0	<0.001
	MD	<0.001	5.7 ^a \pm 0.8	6.1 ^a \pm 0.8	6.6 ^b \pm 0.8	5.7 ^a \pm 0.7	<0.001
pH	IP		7.4 ^a \pm 0.3	7.2 ^b \pm 0.3	7.1 ^b \pm 0.2	7.2 ^b \pm 0.3	<0.05
	SJ		7.2 ^a \pm 0.2	7.0 ^b \pm 0.2	6.9 ^b \pm 0.2	7.0 ^b \pm 0.3	<0.001
	MD	<0.001	7.2 ^a \pm 0.3	7.1 ^{ab} \pm 0.2	6.9 ^b \pm 0.4	7.1 ^{ab} \pm 0.3	<0.05
EC (μ S.cm ⁻¹)	IP		54.8 ^a \pm 4.9	55.6 ^a \pm 10.3	51.0 ^b \pm 5.2	50.9 ^b \pm 7.1	>0.05
	SJ		88.6 ^a \pm 15.1	92.9 ^a \pm 23.3	84.5 ^a \pm 19.0	81.4 ^a \pm 17.1	>0.05
	MD	<0.001	76.3 ^a \pm 10.4	83.2 ^a \pm 14.7	79.7 ^a \pm 15.5	76.6 ^a \pm 14.0	>0.05
N-NH ₃ (μ g.L ⁻¹)	IP		141.7 ^a \pm 73.1	143.0 ^a \pm 106.9	156.0 ^a \pm 129.3	197.3 ^a \pm 195.1	>0.05
	SJ		911.0 ^a \pm 406.1	877.5 ^a \pm 592.8	802.6 ^a \pm 331.1	769.7 ^a \pm 402.4	>0.05
	MD	<0.001	628.2 ^a \pm 264.8	737.6 ^a \pm 398.1	876.6 ^a \pm 508.5	759.1 ^a \pm 403.9	>0.05
N-NO ₂ (μ g.L ⁻¹)	IP		5.4 ^a \pm 5.6	5.6 ^a \pm 4.8	4.7 ^a \pm 3.0	4.7 ^a \pm 2.3	>0.05
	SJ		26.0 ^a \pm 16.0	24.3 ^a \pm 13.7	18.8 ^a \pm 12.7	24.0 ^a \pm 11.5	>0.05
	MD	<0.001	38.0 ^a \pm 75.8	69.6 ^a \pm 244.6	20.1 ^a \pm 8.7	23.6 ^a \pm 11.3	>0.05
N-NO ₃ (μ g.L ⁻¹)	IP		375.2 ^a \pm 174.1	464.4 ^a \pm 324.5	524.9 ^a \pm 301.3	488.5 ^a \pm 228.3	>0.05
	SJ		382.6 ^a \pm 186.8	502.3 ^{ab} \pm 356.8	613.2 ^b \pm 324.2	503.6 ^{ab} \pm 243.0	<0.05
	MD	>0.05	406.4 ^a \pm 176.3	507.3 ^a \pm 372.6	609.9 ^a \pm 369.7	520.1 ^a \pm 248.8	>0.05
SRP (μ g.L ⁻¹)	IP		28.9 ^a \pm 34.4	32.3 ^a \pm 35.6	32.8 ^a \pm 39.5	41.3 ^a \pm 38.4	>0.05
	SJ		65.3 ^a \pm 54.6	77.2 ^a \pm 59.1	71.8 ^a \pm 56.2	77.8 ^a \pm 51.3	>0.05
	MD	<0.001	48.9 ^a \pm 38.1	113.4 ^a \pm 24.3	70.3 ^a \pm 53.1	85.2 ^a \pm 55.1	>0.05
Chl-a (μ g.L ⁻¹)	IP		3.5 ^a \pm 0.4	1.5 ^b \pm 1.3	1.0 ^c \pm 0.8	2.8 ^d \pm 2.7	<0.001
	SJ		7.5 ^a \pm 7.3	3.0 ^b \pm 3.2	2.4 ^c \pm 2.8	3.6 ^b \pm 2.4	<0.001
	MD	<0.001	7.6 ^a \pm 6.2	7.5 ^b \pm 16.7	2.6 ^b \pm 4.6	2.8 ^b \pm 2.2	<0.001

With respect to seasonal limnological variation (Table 1), there were significant differences between the average temperatures detected in the four seasons at site IP. The autumn and spring average T values were significantly different on the sites IP and SJ. On the other hand, the average value of Z was significantly lower in summer for the three sites. The Z_{eu}/Z ratio (%) was not significantly different between summer and autumn for the IP and MD sites. Similar to the seasonal average temperatures at site IP, DO showed values that were significantly higher in winter than summer. At the SJ site, the average dissolved oxygen in spring was not significantly different from that in the other seasons, whereas at the MD site, the average DO was significantly higher only in winter. The average pH values were substantially and significantly higher in summer for the three sites, whereas the variables EC, N-NH₃, N-NO₂, N-NO₃ and SRP showed almost no significant differences in terms of the average seasonal variations, except that EC was significantly lower in summer at the IP site, and N-NO₃ was significantly lower in summer compared to winter at the SJ site. The average values of Chl-a presented a directly proportional correspondence to temperature and an inversely proportional correspondence to DO at site IP, whereas at the SJ and MD sites, only the summer average values of Chl-a were significantly higher in relation to other seasons.

3.2. Phytoplankton

Two hundred and fourteen *taxa* of planktonic algae and cyanobacteria were inventoried, with one hundred ninety-eight being identified to the genus level and the others to the suprageneric level (7.4%). The most representative taxonomic classes in terms of richness were Chlorophyceae (30.8%) and Bacillariophyceae (24.8%), followed by Cyanophyceae (13.6%), Chrysophyceae (9.3%), Euglenophyceae (7.0%) and Zygnematophyceae (6.5%). Cryptophyceae represented 2.3% of the observed richness, while the other classes each contributed 1.4% of the richness (Dinophyceae, Prasinophyceae, Raphidophyceae and Xanthophyceae). The survey quantified one hundred eighty-four *taxa* for site IP, one hundred seventy-nine for site MD and one hundred sixty-five *taxa* for site SJ (Figure 2). The richness of class Chlorophyceae was slightly higher at the IP and MD sites in relation to the SJ site. On the other hand, the absolute and relative contribution of Chrysophyceae was lower, while the relative

contribution of Bacillariophyceae was higher at SJ in relation to the IP and MD sites. When the average sampled richness was considered, rather than the cumulative results of the inventory, it was found to be significantly higher at the SJ and MD sites than at site IP ($p < 0.001$). For all samples, the average density at site IP was 354 ind.mL⁻¹, which was significantly lower than the average density at SJ (732 ind.mL⁻¹) and MD (609 ind.mL⁻¹). The inferential tests confirmed that the average phytoplanktonic richness at site IP was significantly higher in summer compared to the other seasons (Figure 2). At the SJ site, the average richness in autumn and winter was significantly lower than the richness in spring and summer, while at the MD site, it was only possible to confirm that the richness in autumn was significantly lower than the richness in summer. In turn, the average summer densities at the IP and SJ sites were significantly higher compared to the other seasons, while at site MD, it was only possible to conclude that the winter densities were significantly lower than the densities in the other seasons.

The most representative *taxa* belonged to seven taxonomic classes (Table 2). The IP site stood out due to the major contributions of Bacillariophyceae genera (*Aulacoseira* sp., *Cocconeis* sp., *Gomphonema* sp., *Pinnularia* sp., unidentified diatoms), in the density of phytoflagellates from the Cryptophyceae (*Cryptomonas* sp.) and Euglenophyceae (*Euglena* sp., *Strombomonas* sp., *Trachelomonas* sp.) classes and based on the higher density of phytoflagellates from class Chlorophyceae, such as *Eudorina* sp., *Spermatozopsis* sp. (*Spermatozopsis exsultans* Korshikov) and colourless flagellates of class Chrysophyceae (*Cladomonas* sp., *Rhipidodendron* sp.).

The SJ and MD sites stood out in showing the lowest relative contribution among the identified genera of Bacillariophyceae, except for *Asterionella* sp. (*Asterionella formosa* Hassal), in the higher relative contribution of Chlorophyceae (*Actinastrum* sp., *Dictyosphaerium* sp., *Micractinium* sp., *Monoraphidium* sp., *Scenedesmus* / *Desmodesmus* sp.) and due to the higher density of Cryptophyceae and Euglenophyceae. The genera *Cyclotella* sp. and *Nitzschia* sp. as well as phytoflagellates of different classes, such as Peridinales NI, *Bicosoeca* sp., *Chromulina* sp. *Synura* sp. and *Rhodomonas* sp. (*Rhodomonas minuta* v. *nannoplanctica* Skuja), were more expressive at the MD site in relation to SJ and IP sites.

The highest phytoplankton density (Figure 3) generally occurred in January (summer), while

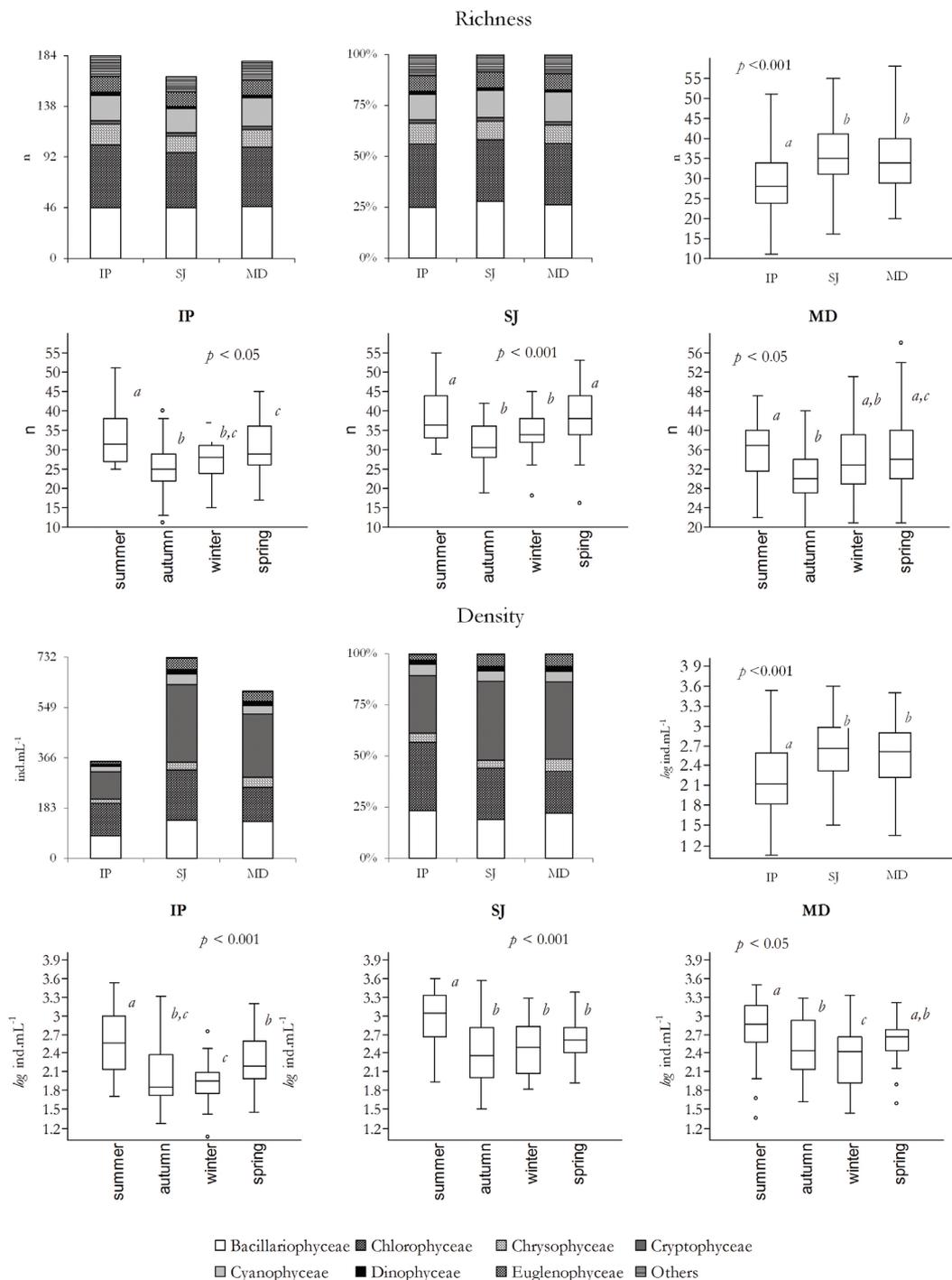


Figure 2. Absolute and relative contributions of taxonomic classes and sites and seasonal variations of richness and density of phytoplankton in Guaiba Lake; different letters indicate significant differences of values at $p < 0.05$.

the lowest density occurred in July (winter) within the annual cycles. Almost all of the most representative taxonomic classes experienced an increase in density towards summer in the annual cycles, whereas the most representative groups in winter were Bacillariophyceae and Cryptophyceae. Some density increments, which were generally less

than the annual summer increases, were observed in spring at all sites. In 2000, a considerable contribution of Bacillariophyceae at site SJ it was observed in late winter (September), when the density of diatoms was 1,863 ind.mL⁻¹, mainly contributed by *Asterionella sp.*, and there were low increments of density between winter and spring.

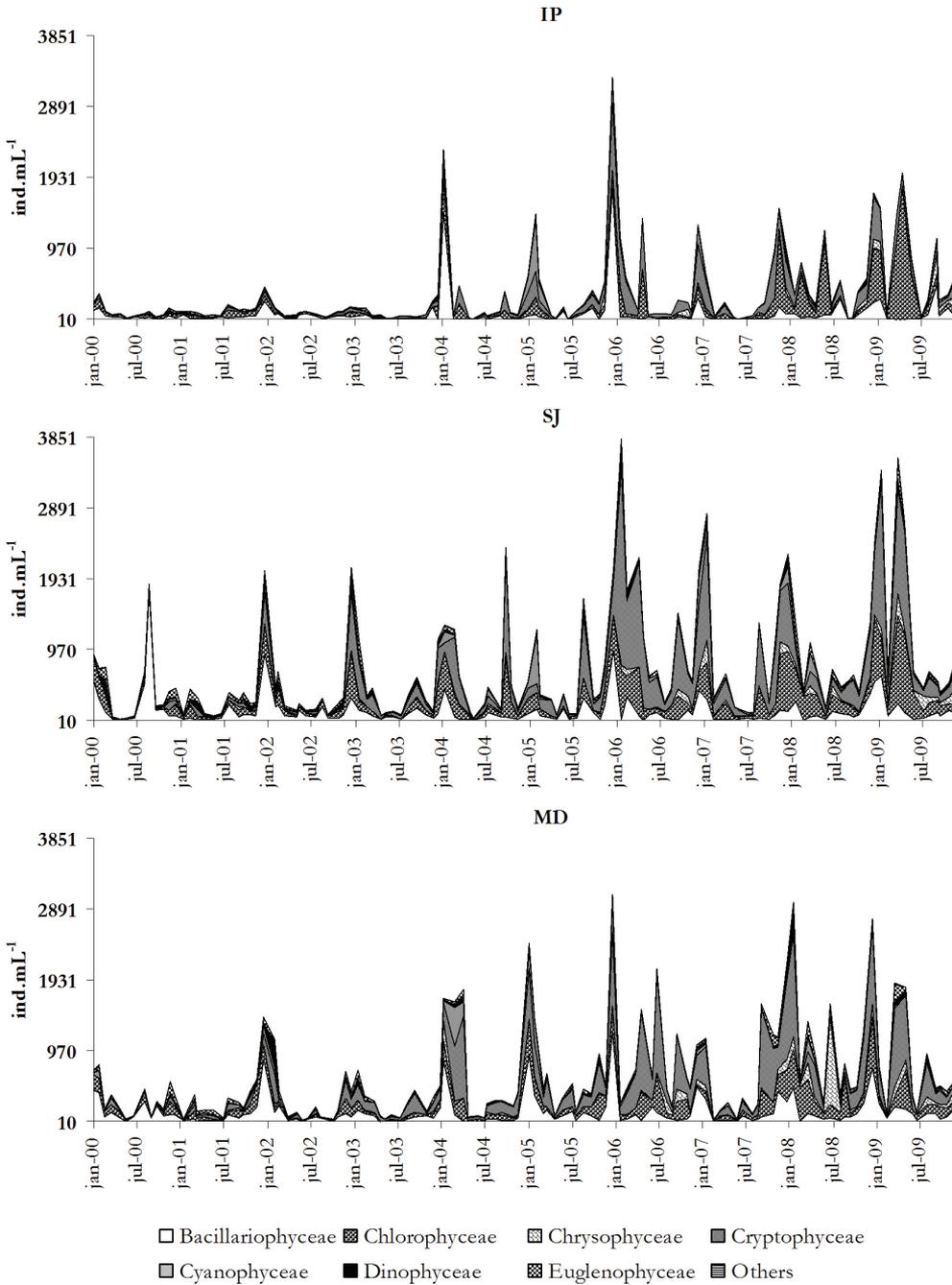


Figure 3. Temporal profile of density classes of phytoplankton in Guaiba Lake.

In that year, *Asterionella sp.* represented 70% of the phytoplanktonic density (283 ind.mL⁻¹) at the MD site.

In the summer months and early fall of 2004, there was a greater contribution of cyanobacteria belonging to the taxa *Planktothrix/Planktothricoides*, i.e., *P. isoethrix* (Skuja) Komárek & Komárková and *P. agardhii* (Gomont) Anagnostidis & Komárek as well as *Planktothricoides raciborskii* (Woloszynska) Suda & Watanabe at sites SJ and MD, and in 2005, a similar situation was observed at sites IP and SJ.

In January 2007, cyanobacteria also contributed to the phytoplankton densities at the three sites, and in 2008, the contribution of cyanobacteria persisted until early autumn at the MD and SJ sites.

3.3. Relationship between phytoplankton and limnological variables

The PCA accounted for 85.5% of the variation in the analysed system (Figure 4). The first axis (56.9%) represented the longitudinal distribution of sites, and it was positively and significantly related

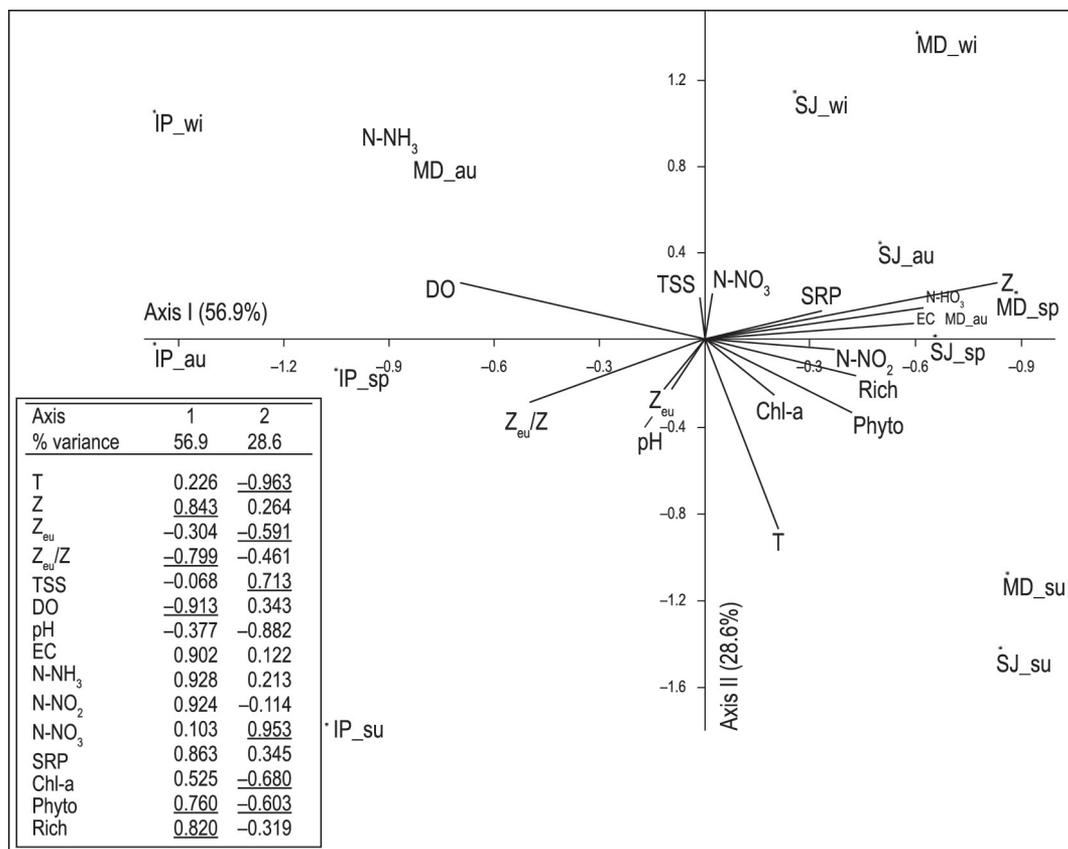


Figure 4. Biplot of seasonal units at the IP, SJ and MD sites and variable vectors on the first two PCA axes; su – summer; sp – spring; wi – winter; au – autumn; in box: underlined numbers $p < 0.05$, bold numbers $p < 0.001$.

to EC, N-NH₃, N-NO₂ and SRP, with higher concentrations of Chl-a being associated with a higher density and richness of phytoplankton at the SJ and MD sites, regardless of the season. On the negative side, the first axis was correlated with shallower and more transparent waters, together with lower DO and Chl-a concentrations as well as a lower richness and density of phytoplankton at site IP. The second axis (28.6%) represented the seasonal variation in limnological variables and the density and richness of phytoplankton. The temperature increase toward the negative side of axis 1 was associated with increased pH and Chl-a values as well as the density and richness of phytoplankton towards the summer months for the three sites considered. At the three sites, the winter season was mainly characterised by low temperatures and higher levels of TSS and DO.

Regarding the composition of genera, it was found that pennated diatoms of the genera *Asterionella sp.* ($r = -0.166$), *Encyonema sp.* ($r = -0.116$), *Eunotia sp.* ($r = -0.130$), *Gomphonema sp.* ($r = -0.187$) and *Pinnularia sp.* ($r = -0.161$) were significantly correlated ($p < 0.05$) with low temperatures, and

among these genera, *Asterionella sp.* was associated with higher levels of SRP ($r = 0.427$), which were observed at the MD and SJ sites compared to site IP (Table 2). Centric diatoms (*Aulacoseira sp.*, $r = 0.441$; *Cyclotella sp.*, $r = 0.367$) and stellate filaments of *Nitzschia sp.* (*Nitzschia fruticosa* Husted; $r = 0.309$) were associated with elevated temperatures. Among the observed phytoflagellates, the genera *Trachelomonas sp.* and *Euglena sp.* (Euglenophyceae) were correlated with the deeper waters ($r = 0.246$ and 0.306), rich in N-NH₃ ($r = 0.342$ and 0.381), with high EC ($r = 0.295$ and 0.395) and low DO levels ($r = -0.472$ and -0.398). Between green flagellates, *Chlamydomonas sp.* ($r = 0.350$), *Eudorina sp.* ($r = 0.365$) and *Pandorina sp.* ($r = 0.367$) were positively associated with temperature. However, only *Chlamydomonas sp.* were more associated with Z_{cu} ($r = 0.265$) and higher EC ($r = 0.302$) and Chl-a values ($r = 0.336$). *Spermatozopsis exsultans* was most strongly associated with a greater Z_{cu} ($r = 0.582$). The most representative genera in terms of biomass (Chl-a) were *Aulacoseira sp.* ($r = 0.256$) and *Cyclotella sp.* ($r = 0.250$), *Monoraphidium sp.* ($r = 0.204$), *Chlamydomonas sp.*

Table 2. Average relative contribution (%) of descriptive *taxa* to the total density of phytoplankton at the IP, SJ and MD sites in Guaiba Lake; NI – unidentified.

BACILLARIOPHYCEAE	IP	SJ	MD
<i>Asterionella sp.</i>	<0.1	3.1	0.8
<i>Aulacoseira sp.</i>	7.8	4.0	5.7
<i>Cocconeis sp.</i>	0.3	0.2	0.1
<i>Cyclotella sp.</i>	3.9	3.2	5.4
<i>Encyonema sp.</i>	0.1	0.1	0.1
Pennate diatom NI	1.3	0.6	0.8
<i>Eunotia sp.</i>	0.1	0.1	0.1
<i>Gomphonema sp.</i>	1.0	0.5	0.6
<i>Melosira sp.</i>	0.6	0.3	0.4
<i>Navicula sp.</i>	1.0	0.8	1.0
<i>Nitzschia sp.</i>	5.8	5.5	6.0
<i>Pinnularia sp.</i>	0.3	0.2	0.2
CHLOROPHYCEAE			
<i>Actinastrum sp.</i>	0.1	0.5	0.4
<i>Ankistrodesmus sp.</i>	0.3	0.6	0.3
<i>Chlamydomonas sp.</i>	7.6	8.1	7.1
<i>Crucigenia sp.</i>	0.4	0.4	0.6
<i>Dictyosphaerium sp.</i>	0.2	0.8	0.6
<i>Eudorina sp.</i>	0.4	0.2	0.2
<i>Golenkinia sp.</i>	0.2	0.1	0.1
<i>Micractinium sp.</i>	0.2	1.3	1.1
<i>Monoraphidium sp.</i>	2.6	3.4	3.2
<i>Oocystis sp.</i>	<0.1	0.2	0.2
<i>Pandorina sp.</i>	0.2	0.3	0.2
<i>Pedinopera sp.</i>	1.0	0.7	0.5
<i>Scenedesmus/Desmodesmus sp.</i>	1.6	2.5	1.8
<i>Spermatozopsis sp.</i>	17.9	4.6	3.5
DINOPHYCEAE			
Peridinales NI	2.1	2.3	2.5
CHRYSOPHYCEAE	IP	SJ	MD
<i>Bicosoeca sp.</i>	2.2	1.9	2.3
<i>Chromulina sp.</i>	0.2	0.2	0.4
<i>Chrysococcus sp.</i>	0.1	0.3	0.2
<i>Cladomonas sp.</i>	0.4	0.1	<0,1
<i>Codosiga sp.</i>	0.4	0.1	0.4
<i>Dinobryon sp.</i>	0.1	0.1	0.1
<i>Mallomonas sp.</i>	0.7	0.8	0.8
<i>Rhipidodendron sp.</i>	0.3	<0.1	<0.1
<i>Synura sp.</i>	0.1	0.4	1.7
CRYPTOPHYCEAE			
<i>Chroomonas sp.</i>	0.7	0.3	0.7
<i>Cryptomonas sp.</i>	27.4	38.3	37.0
<i>Rhodomonas sp.</i>	9.3	8.6	13.1
CYANOPHYCEAE			
<i>Anabaena sp.</i>	0.8	0.4	0.3
<i>Merismopedia sp.</i>	0.8	1.4	0.9
<i>Planktothrix/Planktothricoides sp.</i>	3.3	2.2	2.8
<i>Pseudanabaena sp.</i>	0.3	0.5	0.5
EUGLENOPHYCEAE			
<i>Euglena sp.</i>	0.5	1.9	1.7
<i>Phacus sp.</i>	0.1	0.1	0.1
<i>Strombomonas sp.</i>	0.2	0.3	0.3
<i>Trachelomonas sp.</i>	1.8	3.1	3.3

($r = 0.336$), *Mallomonas sp.* ($r = 0.281$), *Cryptomonas sp.* ($r = 0.341$) and dinoflagellates - PeridNI ($r = 0.290$). The higher correlation of Planktothrix/Planktothricoides (*Planktothrix isoetrix* and *Planktothricoides raciborskii*) with Chl-a ($r = 0.509$) was influenced by summer peaks, mainly in 2004 (MD and SJ) and 2005 (IP and SJ).

4. Discussion

The trophic status of a water body can roughly be assessed based on information regarding the concentration of the limiting nutrient, chlorophyll-a and transparency, which indicate algal biomass, sediment resuspension and penetration of light into the water. The most widely accepted limits of these variables have been suggested by OECD (1982) to characterise the trophic categories (oligotrophic, mesotrophic and eutrophic) of inland waters (Istvánovics, 2009). In this study, the ranges of the mean to maximum values of Chl-a at the IP, SJ and MD sites were found to be 2.7-26.0, 4.2-37.6 and 5.0-88.9 $\mu\text{g.L}^{-1}$, respectively. According to OECD (1982), average values between 2.5 and 8.0 $\mu\text{g.L}^{-1}$ characterise mesotrophic waters, while maximum values above 25.0 $\mu\text{g.L}^{-1}$ characterise eutrophic waters. The average SRP values were also higher than the limit of 35.0 $\mu\text{g.L}^{-1}$ of total phosphorus for mesotrophic waters at the SJ and MD sites, characterising these sites as eutrophic. Additionally, the average Secchi disk values ($Z_{\text{eu}}/3$) obtained at the three sites were less than 3.0 m, typical of eutrophic waters.

The lowest average SRP value obtained at the IP site (34.1 $\mu\text{g.L}^{-1}$) was coincident with the lowest average values recorded for EC (53.1 $\mu\text{S.cm}^{-1}$), dissolved inorganic nitrogen (mainly in the form of N-NH_3 ; 160.9 $\mu\text{g.L}^{-1}$), Chl-a (2.7 $\mu\text{g.L}^{-1}$) and the total phytoplankton density (354 ind.mL^{-1}). This set of parameter values characterised this site as “less eutrophic” in relation to SJ and MD, which was also corroborated by the higher average DO (7,8 mg.L^{-1}) and Z_{eu}/Z (38.8%) values observed at the IP site in relation to sites SJ and MD. These data and the limits established by the OECD suggest meso-eutrophic conditions for site IP and truly eutrophic conditions for sites SJ and MD in Guaiba Lake.

The trophic statuses of the three sites investigated in this study were associated with their locations in different watersheds. The IP site is influenced by the flow of the Jacuí River, which is the largest tributary to the lake, approximately 1,850 $\text{m}^3.\text{s}^{-1}$, and whose basin is predominantly occupied by

agricultural activities (Noronha, 1998; Faria and Lersch, 2001). The trophic statuses determined at the MD and SJ sites corresponded to the higher occupancy of these basins in terms of population. Thus, the water quality at the latter two sites has become more eutrophic, whether due to higher concentrations of nutrients and chlorophyll-a or the greater density of phytoplankton. This finding corroborates the idea that the structure and development of phytoplankton are influenced by the hydrology and the use and occupation of the corresponding watershed, as observed in the inland waters in tropical zones of Brazil (Huszar, 1996).

In this study different taxonomic classes of phytoplanktonic algae had significantly higher density in eutrophic points (MD and SJ) compared to meso-eutrophic point, as well as, it had significantly higher density in the summer compared to other seasons. This finding revealed that the traditional classification of phytoplankton taxonomic may be a limited tool in limnological assessment of lakes and rivers. Reynolds et al. (2002) has proposed of a functional classification scheme for the phytoplankton species that has been tested and it is considered as an important predictive tool. This classification scheme brings species with similar morphology, physiology, and ecology together into functional groups named using alphanumerical characters. Since then, tests using phytoplankton have been constantly improved to qualify the environments (Padisák et al., 2006).

Regarding the composition of phytoplankton, diatom genera, such as *Cocconeis sp.*, *Gomphonema sp.*, *Pinnularia sp.* and unidentified diatoms, referred to as DiaPenNI, showed a higher relative contribution at the IP site in relation to the others. According to Padisák et al. (2009), some pennated diatoms belonging to the MP functional group are indicators of river flows, which agrees with the hydrodynamic characterisation of site IP, which is influenced by the greatest river flow. In contrast, the genus *Asterionella sp.* showed a higher mean density at sites SJ and MD. This exception agrees with studies showing that blooms of *A. formosa* are associated with higher phosphorus availability in certain periods of seasonal variation, especially the vernal period in temperate zones of the Earth (Sommer, 1988). *A. formosa* belongs to the C functional group, indicating eutrophic small and medium-sized lakes that are sensitive to the onset of stratification (Padisák et al., 2009).

Despite exhibiting significantly higher average TSS (22.9 mg L^{-1}), EC ($86.8 \text{ } \mu\text{S.cm}^{-1}$) and N-NH₃

($853.6 \text{ } \mu\text{g.L}^{-1}$) values, the SJ site showed no significantly different values regarding Chl-a or the richness and density of phytoplankton in relation to the MD site. The SJ site is located downstream from the mouth of the Gravataí River, which is a tributary presenting a lowest flow rate (approximately $59 \text{ m}^3.\text{s}^{-1}$), highest human population density and highest concentrations of pollutants in the region (Noronha, 1998). The apparently most polluted condition observed at the SJ site in relation to the MD and IP sites coincided with the greatest relative contribution of Chlorophyceae genera such as *Actinastrum sp.*, *Ankistrodesmus sp.*, *Dictyosphaerium sp.*, *Micractinium sp.*, *Monoraphidium sp.*, and *Scenedesmus/Desmodesmus sp.* and the greater relative contribution of phytoflagellate genera belonging to class Cryptophyceae (*Cryptomonas sp.*) and Euglenophyceae (*Euglena sp.*) to the density of phytoplankton. Unicellular and coenobial/colonial green algae belonging the F, X₁ and J functional groups are associated with shallow eutrophic systems (Padisák et al., 2009), while cryptomonads (X₂ and Y groups) are linked to the availability of nutrients (Klaveness, 1988) and euglenoids (W₁ group) to the availability of organic matter (Tell et al., 2005).

The increasing values of Chl-a, phytoplankton density, EC, and some forms of nutrients at the IP, MD and SJ sites coincided with the water quality index (WQI) values observed over time in a long-term study (2000-2009) by Andrade et al. (2012), in which the mean scores (and ranges) were found to be 69.8 (regular), 51.3 (regular/bad) and 49.2 (bad) for these sites, respectively. This comparison confirmed the sensitivity of the composition and density of phytoplankton in indicating different trophic conditions as well as the different water quality conditions observed at the sites investigated in the present study.

For the three sites, there were higher relative densities of the genera *Aulacoseira sp.*, *Chlamydomonas sp.*, *Spermatozopsis sp.*, *Cryptomonas sp.* and *Rhodomonas sp.* According to Reynolds (2006), *Aulacoseira* species (P group, *Aulacoseira granulata* v. *angustissima* (O. Müller) Simonsen) are meroplanktonic (spending one phase of life in the sediment and another in the pelagic zone) and can be significant in shallow lakes under meso-eutrophic conditions when exposed to wind and wave action, as occurs in the investigated lake (Nicolodi et al., 2010). Nano-microplanktonic flagellates (X₂ and Y groups) belonging to the genera mentioned above are collectively common and constitute

representative assemblages in shallow lakes whose main strategy is rapid growth.

The high diversity of Chlorophyceae and Bacillariophyceae at the three sites was in accord with the findings of a short-term study showing that say these groups are more representative of the diversity observed at the mouths of the rivers that form the delta of the Jacuí River, upstream of Guaíba Lake (Rodrigues et al., 2007).

Long-term studies have been performed to establish seasonal patterns in phytoplankton and to verify variations over the years due to hydrological, nutritional and climatic changes (Anneville et al., 2004). In this study, the long-term data obtained were useful for verifying significant seasonal patterns in limnological conditions as well as phytoplankton richness and density. If the temperature in the tropical zone is not a limiting factor for the development of phytoplankton (Huszar, 1996), the same cannot be said for Guaíba Lake, which is situated in the subtropical zone of Brazil, considering that the average density in winter recorded in this long-term study was significantly lower than the average density in summer.

The significantly higher temperatures in summer coincided with significantly higher values of Chl-a, pH, phytoplankton density and Z_{cu}/Z (%), and significantly lower values of Z, TSS and DO. The increased water temperature in summer is related to the more intense solar radiation that occurs in the subtropical region of Brazil in this season (Mendonça and Danni-Oliveira, 2007) because the variation in irradiance is cyclical and is inversely proportional to latitude. This pattern characterises temperature as a determining factor in the seasonal abundance and the distribution and geographic variation of phytoplankton (Reynolds, 1988). Because light availability is crucial to the development of phytoplankton, some classes of algae experienced a density increase in summer in Guaíba Lake, and only seven genera were negatively correlated with water temperature, five of which were pennated diatoms (*Asterionella* sp. *Encyonema* sp. *Eunotia* sp. *Gomphonema* sp. *Pinnularia* sp.), and two were colonial flagellates of class Chrysophyceae (*Cladomonas* sp. *Synura* sp.).

Regarding the reduced Z in the lake in summer, the long-term study by Andrade and Giroldo (2010) revealed a significant correlation of Z with the volume of rain. The authors stated that the thermal/climatic seasonality of Guaíba Lake is accompanied by hydrological seasonality and that summer is the

season showing the lowest values of accumulated precipitation and depth.

In contrast to what has been demonstrated in subtropical reservoirs (Rodrigues et al., 2005), the development of phytoplankton in Guaíba Lake in summer was not followed by an increase in TSS, but by increased transparency. Similar behaviour has been observed in subtropical Brazilian rivers, where abiogenic turbidity during rainy periods in winter and spring overcomes the biogenic turbidity caused by the development of phytoplankton in summer (Devercelli, 2006).

The temporal profile of the phytoplanktonic density by class shows that the peak densities of Chlorophyceae, Bacillariophyceae (centric diatoms: *Aulacoseira* sp., *Cyclotella* sp.) and Cryptophyceae in summer were most likely associated with low water levels subjected to turbulence caused by winds, as usually occurs in the lake (Nicolodi et al., 2010). The expressive contribution of cyanobacteria, especially in 2004 (MD and SJ sites) and 2005 (IP and SJ sites), characterised by development of *Planktothrix isothrix* and *Planktothricoides raciborskii* were associated with anomalies in precipitation (*La Niña*) and high values of chlorophyll-a ($r = 0.509$, $p < 0.01$) according to Andrade and Giroldo (2010) as well as high transparency and temperatures (Maizonave et al., 2009). According to Padišák et al. (2009) *P. isothrix* (referred to as *P. mougeotii*) belongs to R codon. Such assemblages are associated with stratification and occur in the metalimnion or upper hypolimnion of oligomesotrophic lakes. Because lower phosphorus levels occur in the summer and the main source of this element during periods of high hydraulic retention (due to low rainfall in anomalous periods) should be the sediment, it is likely that these species (*P. isothrix* and *Planktothricoides raciborskii*) explore the bottom to assimilate and store phosphorus (Paerl, 1988). In fact, short term study conducted by Ribeiro et al. (2012) in a south bay of Guaíba Lake, between spring 2011 and summer 2012 has suggested that changes in rainfall patterns due to *La Niña* can influence the environment, increasing transparency and recruiting cyanobacterial inocula deposited in layers more enriched. The study also pointed out that there was thermal stratification prior to the beginning of summer *Planktotricoides raciborskii* bloom.

The antagonistic conditions in winter confirmed that low temperatures and high turbidity (high TSS) inhibit the development of phytoplankton in Guaíba Lake, which confirms that there are seasonal

limnological conditions in this lake, associated with variations in phytoplankton.

The results of this study show the potential of phytoplankton regarding differentiating between trophic state conditions and, thus, for assisting in the limnological characterisation of inland waters. The composition and density of phytoplankton should also be helpful in diagnosing the quality of waters that are subject to eutrophication and the development of cyanobacteria, as occurs in many lakes and rivers in Brazil (Abe et al., 2006; Sant'anna et al., 2008). The subtropical inland waters in the subtropical region of Brazil suffer abnormal rain fluctuations related to the *El Niño* southern oscillation (Abreu et al., 2010; Andrade and Giroldo, 2010). The occurrence of this type of pattern alters the seasonal fluctuations of phytoplankton, corroborating the significance of long-term studies to better characterise and understand phytoplankton ecology.

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