Iheringia
Série Botânica
ISSN ON-LINE 2446-8231

Jardim Botânico de Porto Alegre
Museu de Ciências Naturais

Morphological, ecological and toxicological aspects of *Raphidiopsis raciborskii* (Cyanobacteria) in a eutrophic urban subtropical lake in southern Brazil

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Recebido em 05.VIII.2019
Aceito em 28.VII.2020

DOI 10.21826/2446-82312020v75e2020018

ABSTRACT – A dense perennial bloom of *Raphidiopsis raciborskii* was observed in an urban lake (29°53'13"S and 51°09'29.9"W). Due to supposed toxicity, this study analyzed the morphology of the species, evaluated the main factors that influence its growth, and examined bloom toxicity. Two sites were sampled monthly from November 2009 to November 2010. The species was found in all samples and dominated in 77% of the samples. Highest density was recorded at the end of summer (March – 199,550 ind.mL^{-1}) at 26.6 ºC and pH 7.8. High densities were also found at low temperatures (72,145 ind.mL^{-1} at 12.6 °C and 130,475 ind.mL^{-1} at 14.5 °C) and at minimum (5.4) and maximum (8.7) pH, reaching 89,964 ind.mL^{-1} and 61,400 ind.mL^{-1}, respectively. Nitrogen availability was high, especially ammonium [(60-)140-660 µg.L^{-1}], and phosphorus was low (orthophosphate < 10 µg.L^{-1}). These results support that *R. raciborskii* has a wide tolerance to abiotic variations. Saxitoxins and gonyautoxins were found in the bloom.

Keywords: cyanobacterial bloom, *Cylindrospermopsis raciborskii*, neurotoxins

RESUMO – Aspectos morfológicos, ecológicos e toxicológicos de *Raphidiopsis raciborskii* (Cyanobacteria) em um lago subtropical urbano e eutrófico no extremo sul do Brasil. Densa e perene floração de *Raphidiopsis raciborskii* foi observada em um lago urbano (29°53'13"S e 51°09'29,9"W). Devido à suposta toxicidade, esse estudo analisou a morfologia da espécie, avaliou os principais fatores que interferem no seu crescimento e examinou a toxicidade da floração. Dois locais foram amostrados mensalmente entre novembro de 2009 e novembro de 2010. A espécie foi encontrada em todas as amostras, dominante em 77%. A maior densidade foi registrada no final do verão (março - 199.550 ind.mL^{-1}) a 26,6 ºC e pH 7,8. Altas densidades também foram observadas sob baixas temperaturas (72.145 ind.mL^{-1} a 12,6° C e 130.475 ind.mL^{-1} a 14,5° C) e no pH mínimo (5,4) e máximo (8,7) registrado, atingindo 89.964 ind.mL^{-1} e 61.400 ind.mL^{-1}, respectivamente. A disponibilidade de nitrogênio foi alta, especialmente amônio [(60-)140-660 µg.L^{-1}], e a de fósforo foi baixa (ortofosfato < 10 µg.L^{-1}). Esses resultados sustentam que *R. raciborskii* tem uma ampla tolerância a variações abióticas. Saxitoxinas e goniautoxinas foram detectadas na floração.

Palavras-chaves: *Cylindrospermopsis raciborskii*, florações de cianobactérias, neurotoxinas

INTRODUCTION

Raphidiopsis raciborskii (Woloszynska) Aguilera, Berrendero, Gómez, Kaštovský, Echenique & Salerno is synonymous to *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju was originally described as *Anabaena raciborskii* by Woloszynska (1912) from plankton of a small tropical lake in Java (Indonesia). The species was transferred to *Anabaenopsis* Miller 1923 [=*Anabaenopsis raciborskii* (Woloszynska) Elenkin] due to terminal heterocytes, and later Seenayya & Subba Raju (1972) transferred it to *Cylindrospermopsis* due to the origin of apical heterocytes. Thus, *Cylindrospermopsis* was defined based on the heterocyte origin and position, while the genus *Raphidiopsis* Fritsch & Rich 1929 lacked heterocytes (Komárek & Anagnostidis 1989). However, Komárková (1998) mentioned that due to heterocytes and akinetes usually being rare, sterile trichomes of *Cylindrospermopsis* were easily confused with *Raphidiopsis*. Using a 16S rRNA gene phylogeny, Moustaka-Gouni *et al.* (2009) proposed that *R. mediterranea* from Lake Kastoria, Greece (type locality) was actually a non-heterocytous stage of *C. raciborskii.* Aguilera *et al.* (2018) found that strains of *Raphidiopsis* and *Cylindrospermopsis* were within a

single clade, and not genetically different using both 16S rRNA gene phylogeny and the secondary structure of the 16S-23S rRNA internal transcribed spacer (ITS). Due to the principle of priority, these authors synonymized the genus into *Raphidiopsis* Fritsch & Rich emend. Aguilera, Gómez, Kăstovský, Echenique & Salerno.

Raphidiopsis raciborskii (= *Cylindrospermopsis raciborskii*) is a planktonic cyanobacterium known worldwide for forming cyanobacterial harmful algal blooms (cyanoHABs). These cyanoHABs have had chronic and toxic effects, including the death of fish, humans and other animals (Codd & Bell 1984, Falconer & Fitzgerald 1999). This cyanobacterium is increasingly found in tropical and temperate environments of both the Northern and Southern Hemispheres and is considered exotic and invasive (McGregor & Fabbro 2000, Briand *et al.* 2004, Jones & Sauter, 2005, Kokociński *et al.* 2010, Antunes *et al.* 2015, Babanazarova *et al.* 2015). The species is on the list of invasive plants/algae in some states within the U.S. (*e.g.* Indiana and Wisconsin), where dedicated remediation efforts for its control are established. It is common in fresh waters, but also occurs in swamps and slightly brackish waters (1.5-2‰) (Padisák 1997, Antunes *et al.* 2015). In the 1990's, there was an expansion of the species throughout Brazil due to eutrophication (Tucci & Sant'Anna 2003), and it is now ubiquitous in both tropical and subtropical regions of the country. Most work on this species (including toxicology) is from blooms of Brazilian waters (*e.g.* Lagos *et al.* 1999, Bouvy *et al.* 2000, Molica *et al.* 2002, Costa *et al.* 2006, Gemelgo *et al.* 2008, Hoff-Risseti *et al.* 2013).

Since *R. raciborskii* is ecologically and toxicologically important, information on its dense bloom structures in lakes used for recreation or as a resting/breeding area for wildlife is fundamental for proper management. Therefore, we analyzed the morphological variability of the species, temporal and spatial variation of its density, key factors for its development, and bloom toxicity, in order to attain a better understanding of this species.

MATERIALS AND METHODS

Study area

The studied lake (29°53'13"S and 51°09'29.9"W) is the main water body at the Lutheran University of Brazil (ULBRA) located in Canoas, Rio Grande do Sul, southern Brazil. It was constructed about 20 years ago in a shallow area with stones, gravel and rubble and covers 10,150 m^2 with a mean depth of 3.5 m. The lake is supplied by rainwater and runoff from gutters and roofs (via underground pipes), and its east bank is shaded by shoreline trees [*Inga uruguensis* Hook et Arn., *Senna multijuga* (LC Rich.) Irwin & Barn., *Psidium cattleianum* Sat., and *Pittosporum undulatum* Vent.]. Turtles, fish and birds are found in the lake and there is a small island that is a resting and breeding area for wildlife. This lake is in a place used for recreation and leisure due to its scenic beauty and central location (Figure 1).

Sampling

Collections were carried out monthly between November 2009 and November 2010, at two sites, one at the inflow of the water (I) and the other at the outflow (O) (n=26) (Figure 1). For taxonomic analyses, samples were taken with a plankton net (30 μm) and for quantitative counts the surface of the water was sampled via a grab sample; these were fixed with 4% formaldehyde and Lugol's solution, respectively. The samples are deposited in Prof. Dr. Alarich R.H. Schultz Herbarium (HAS) of the Natural Sciences Museum (MCN), Porto Alegre, Rio Grande do Sul state, Brazil, under accession numbers HAS 108645 to HAS 108702. Concentrated subsamples were kept unfixed for the analysis of live material and microphotographs.

Abiotic variables such as pH, water temperature (°C) and air temperature (°C) were measured in the field using a portable potentiometer (EC 10 Model 50050), electrical conductivity ($\mu S.cm^{-1}$) was measured with a conductivity meter (Model YSI 30), and depth (m) and transparency (m) using a Secchi disc. Samples for the analysis of organic matter ($mg.L^{-1}$), ammonium ($\mu g.L^{-1}$), nitrate ($\mu g.L^{-1}$), nitrite ($\mu g.L^{-1}$), orthophosphate ($\mu g.L^{-1}$), dissolved oxygen ($mg.L^{-1}$) and dissolved oxygen saturation (%) were kept refrigerated and analyzed following standard methods (APHA 2005).

Taxonomic analysis

Taxonomic studies were performed using a LEICA DM-LB light microscope and the photographs were taken from the ocular using a digital camera. Quantitative analyses were carried out using an inverted light microscope WILD M40, following Utermöhl (1958). Sedimentation chambers of 10 ml were used, and the sedimentation time was 6 hours. Counts were performed by vertical transects and the limit of counted fields was calculated based on Pappas & Stoermer (1996).

Statistical analysis

We followed the criteria established by Lobo & Leighton (1986) to determine abundance and dominance, where the taxa whose numerical occurrence exceeds the mean number of individuals per species are considered abundant, and dominant are those that are more than 50% of the total number of individuals counted in a sample.

Ordination of abiotic data was carried out using Principal Component Analysis (PCA) (covariance matrix) to determine the variability of environmental data in relation to the study period (Valentin 2000). The analysis was done using PC-ORD version 6 for Windows.

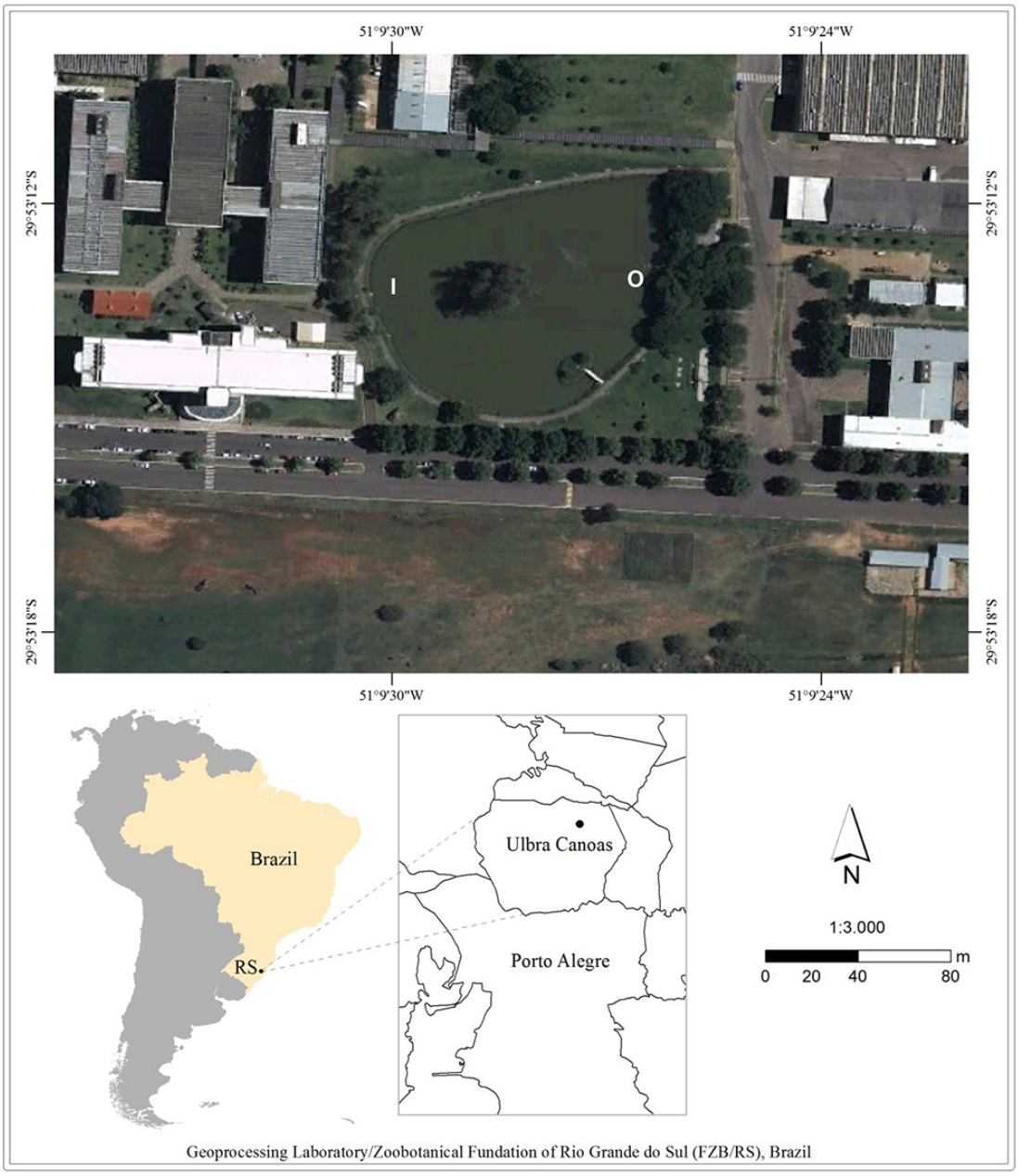


Fig. 1. Map of the studied lake, showing the two sampling sites (I = water inflow and O = water outflow).

Cyanotoxin analysis

Surface samples were collected at two sites to determine cyanotoxins, water inflow (I) and water outflow (O), in November 2010. A 10 ml aliquot of the sample was acidified (pH 2) with 0.05 M hydrochloric acid. Saxitoxins were detected by High Performance Liquid Chromatography with fluorescence detection (HPLC-FLD, Shimadzu, Japan), following the post-column derivatization methodology recommended by Oshima (1995) and compared to standards of seven variants provided by NRC Research Council, Canada.

RESULTS AND DISCUSSION

Community structure

Cyanobacteria were the most abundant group of phytoplankton during the study, with more than 90% of total density in 73% of the analyzed samples (n=26) (Figure 2). *Raphidiopsis raciborskii* was found in all samples with 85% of total community density (2,166,777 ind.mL^{-1}), capable of changing the color of the water to greenish-yellow and yellow. The species was dominant in 77% of the samples and abundant in 15% of the samples. The highest densities

were seen at the end of summer (March 2010: 199,550 ind. mL^{-1}) and in mid-autumn (May 2010: 169,191 ind.mL^{-1}), near the inflow of the water, and the lowest densities were found at the end of spring (December 2009: inflow: 482 ind.mL^{-1} and outflow: 737 ind.mL^{-1}) (Figure 3). At that time, diatoms became more abundant than *R. raciborskii* at both sites (inflow: 24,500 ind.mL^{-1} and outflow: 17,644 ind.mL^{-1}), with *R. raciborskii* representing only 1.5% and 3.2% of total density, respectively.

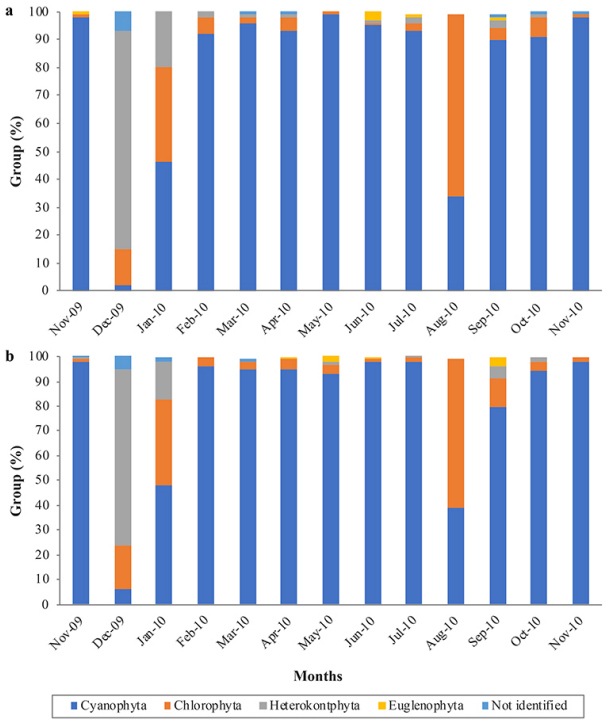


Fig. 2a, b. Bar graphs indicating the relative percentages of different phytoplankton groups in the lake inflow (a) and outflow (b), sampled every month from November 2009 to November 2010.

Chlorophytes were found in high concentrations [inflow: 93,774 ind.mL^{-1} (65.9% of the community); outflow: 111,543 ind.mL^{-1} (62.8 % of the community)] in samples from August 2010. *Monoraphidium irregulare* (G.M.Smith) Komarková-Legnerová was responsible for the high density of chlorophytes, with 91,542 ind.mL^{-1} (inflow) and 108,473 ind.mL^{-1} (outflow). It was dominant while *R. raciborskii* was only abundant, reaching 48,562 ind.mL^{-1} (inflow) and 72,145 ind.mL^{-1} (outflow) (Figs. 2, 3). In samples collected during summer (January 2010), *R. raciborskii* was also abundant (inflow: 6,036 ind.mL^{-1}; outflow: 5,061 ind. mL^{-1}), along with chlorophytes (inflow: 4,891 ind.mL^{-1}; outflow: 3,983 ind.mL^{-1}) and diatoms (inflow: 2,919 ind. mL^{-1}; outflow: 1,742 ind.mL^{-1}) (Figs. 2, 3).

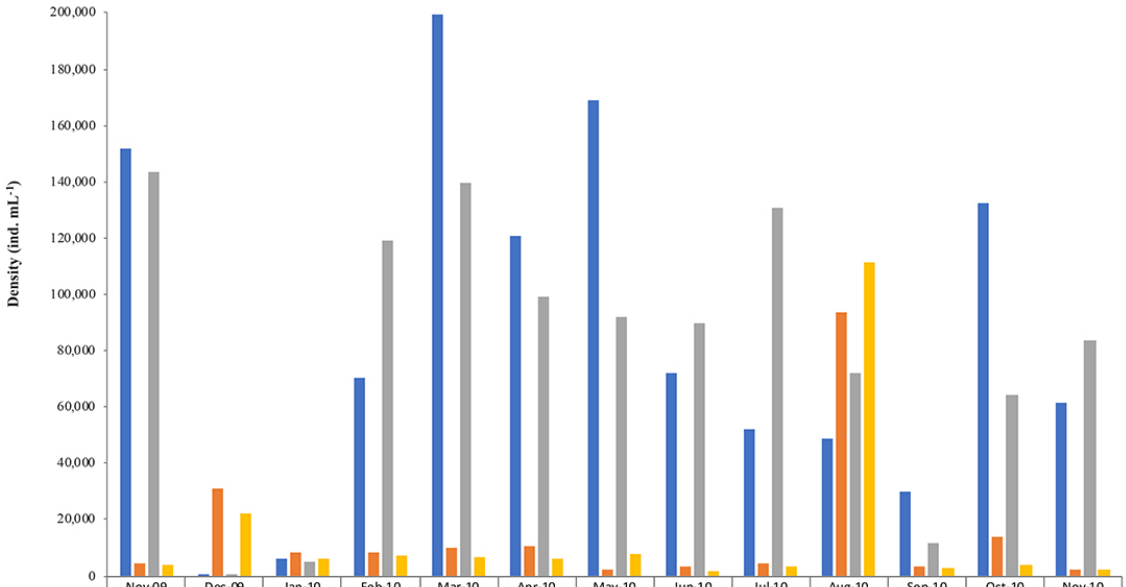
Planktolyngbya limnetica (Lemmermann) Komarková-Legnerová & Cronberg and *Cuspidothrix issatschenkoi* (Usacev) Rajaniemi *et al.* were two other cyanobacteria observed in the *R. raciborskii* blooms at low densities. The highest densities of these two species were found in summer (February 2010) when *P. limnetica* reached 1,316 ind.mL^{-1} (inflow) and 1,535 ind.mL^{-1} (outflow) and *Cu. issatschenkoi* reached 877 ind.mL^{-1} (inflow) and 512 ind. mL^{-1} (outflow). At the same time, *R. raciborskii* was much more abundant with 70,171 ind.mL^{-1} (inflow) and 119,218 ind.mL^{-1} (outflow). *Planktolyngbya limnetica* was present in 58% of our samples, while *Cu. issatschenkoi* was only observed in 19%. Padisák & Reynolds (1998) found similar results while studying the phytoplankton of Lake Balaton (Hungary), where *R. raciborskii* (as *C. raciborskii*) and *Cu. issatschenkoi* co-occurred in 36% of their samples between 1983 and 1997.

The association of *P. limnetica* with the dominance of *R. raciborskii* has also been seen in other Brazilian water bodies, such as the Tabocas Reservoir, Caruaru, Pernambuco (Bressan 2001), Peri Lagoon, Florianópolis, Santa Catarina (Grellmann 2006) and in a lake at Prof Theobaldo Dick Park, Lajeado, Rio Grande do Sul (Hepp 2009). McGregor & Fabbro (2000) found that in Australian reservoirs the abundance of *R. raciborskii* was correlated to other species of non-heterocytous filamentous cyanobacteria [*Limnothrix* aff. *redekei* (Van Goor) Meffert, *Planktolyngbya limnetica* and *P. subtilis* (G.S. West) Anagnostidis & Komárek]. They noted that this had also been observed for the Fitzroy River, Queensland, Australia (Fabbro & Duivenvoorden 1996, Bormans 1999) and in different European lakes (Dokulil & Mayer 1996, Padisák 1997, Padisák & Reynolds 1998). Thus, finding *R. raciborskii* with other species of non-heterocytous filamentous cyanobacteria is common, with reports from temperate, tropical and subtropical environments. Niche overlaps among these species are justified by similar ecological adaptations to conditions such as low light intensity (Vörös 1995) and high levels of ammonium (Présing *et al.* 1996). In the studied lake, the densities of *R. raciborskii* were elevated even at the sampling site shaded by trees along the littoral zone. In 46% of the samples, the density was higher at this location (water outflow) than in the sunny water inflow, and consequently, the number of flakes migrating in the water column increased with a much thicker surface scum. The high densities of *R. raciborskii* recorded at a shaded site of the studied lake demonstrate the ecological adaptation of the species to low light intensity, corroborating the comment of Vörös (1995). Its high densities can also be justified by the excessive nitrogen availability in this site of the lake.

Morphological aspects

Throughout the study, lake water was greenish to yellowish due to the *R. raciborskii* bloom. The species sometimes formed small, yellowish flakes, that migrated in the water column and there was dense surface scum, especially at the littoral zone.

We observed straight and slightly curved trichomes in our samples. No sigmoid or spiral specimens, common in *R. raciborskii* blooms, were seen. Table 1 provides the morphological characteristics and dimensions of the analyzed specimens and figures 4a-c illustrate the specimens.



	Nov-09	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10	Jun-10	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10
Inflow *R. raciborskii*	151,863	482	6,036	70,171	199,550	121,046	169,191	72,285	52,097	48,562	29,773	132,289	61,400
Inflow (other organisms)	4,502	31,062	8,325	8,333	9,824	10,525	2,047	3,631	4,279	93,774	3,128	13,954	2,456
Outflow *R. raciborskii*	143,522	737	5,061	119,218	139,635	99,094	92,100	89,964	130,475	72,145	11,541	64,086	83,504
Outflow (other organisms)	3,837	22,276	6,389	7,164	6,652	6,138	7,535	1,602	3,326	111,543	2,955	3,837	2,511

Fig. 3. Bar graphs indicating the specific densities (ind. ml-1) of *Raphidiopsis raciborskii* and other phytoplankton organisms (Cyanobacteria, Chlorophyta, Heterokontophyta, Euglenophyta and unidentified) sampled from the lake inflow (a) and outflow (b), every month from November 2009 to November 2010.

Table 1. Morphological and metric characteristics [minimum-maximum (mean ± standard deviation)] of *Raphidiopsis raciborskii* populations in the studied lake, from November 2009 to November 2010.

Caracteristics	Trichome	Vegetative cell	Heterocyte	Akinete
Form	Solitary or entangled Straight or slightly curved Slightly or not constricted	Intermediate: cylindrical Apical: pointy, cylindrical, or conical-round	Conical with round ends	Cylindrical with round ends
Width (µm)	-	2-3.5 (2.2±0.6)	2-4 (2.3±0.5)	2-2.5 (3.1±0.6)
Length (µm)	69-177.5 (126.7±34)	2.5-9 (5.7±2.1)	3.8-10.5 (7.7±1.9)	7-14 (11±2.1)
Cell content	-	Always with aerotopes	-	-
Number and position	-	-	Solitary and terminal	Solitary and sub-terminal
Mucilage	Absent	Absent	Absent	Absent

Although *R. raciborskii* is characterized by trichomes with terminal heterocytes (originating from end cells) and subterminal akinetes, the majority of the trichomes in our populations (94.1%) contained only vegetative cells (Fig. 5). Further, only 5.2% (114,027 ind.mL^{-1}) of the populations had heterocytes at one end; no trichomes were observed with heterocytes at both ends. The amount of trichomes with akinetes was also rare; only 0.6% (12,163 ind.mL^{-1}) of those observed were formed by vegetative cells and an akinete, whereas only 0.02% (438 ind.mL^{-1}) had heterocytes and akinetes.

We found trichomes formed by only vegetative cells in all samples (without heterocytes and/or akinetes) and this form was the densest (11,314-190,954 ind.mL^{-1}). In addition, populations collected between November 2009 and January 2010 were exclusively composed of vegetative cells (Fig. 5).

Environmental conditions

Principal Component Analysis (PCA) showed that there was variation in abiotic data over time, but not between the two sampling points. Temporal variation was recorded with the same pattern between the two sampling locations (water inflow and outflow). Samples from the months of July, August, September, October and November 2010 were distributed on the positive side of axis 1, associated with the highest values of organic matter (OM), electrical conductivity (Cond) and pH (Table 2, Fig. 6). The highest

pH was recorded in spring, in October (water inflow) and November 2010 (water inflow and outflow), positive side of axis 1. The sample sites associated to increased water temperature, corresponding to the months of January, February, March, October, and November 2010 are grouped on the negative side of axis 2 (Fig. 6).

During the study, the water temperature fluctuated between 12.6 °C (winter: August 2010) at the outflow and 28 °C (summer: February 2010) at the inflow. The pH varied from acid to alkaline, with a minimum of 5.4 (winter: June 2010) at the outflow and maximum of 8.7 (spring: November 2010) at the inflow. Ammonium concentrations varied considerably with a low of 60 $\mu g.L^{-1}$ (spring: November 2009) at the inflow and outflow and 660 $\mu g.L^{-1}$ (summer: December 2009) at the ouflow. Nitrate concentrations varied between 1,000 to 1,300 $\mu g.L^{-1}$ throughout the study for both sampling. There were large differences in dissolved oxygen throughout the study, with a low of 2.5 $mg.L^{-1}$ (winter: June 2010) at the inflow and high of 10.5 $mg.L^{-1}$ (winter: July 2010) at the ouflow (Table 3).

High temperatures (above 23 ºC) are one of the factors that can increase *R. raciborskii* blooms (eg. Pádisak 1997, Tucci & Sant'Anna 2003, Gemelgo *et al.* 2008). However, Dokulil & Mayer (1996) documented high densities of the species in an Austrian lagoon, where the temperatures did not exceed 18 ºC. Komárková *et al.* (1999) found a correlation between temperature and species forms, where the typical form with cylindrical cells and the characteristic presence of heterocytes developed at higher temperature. According to these authors, the optimum temperature for its growth and development has been found to be 25 ºC. Conversely, anomalous forms (elongate cells, barrel-shaped, filaments with few cells, up to two cells, without heterocytes) occurred at lower temperatures (15-20 ºC).

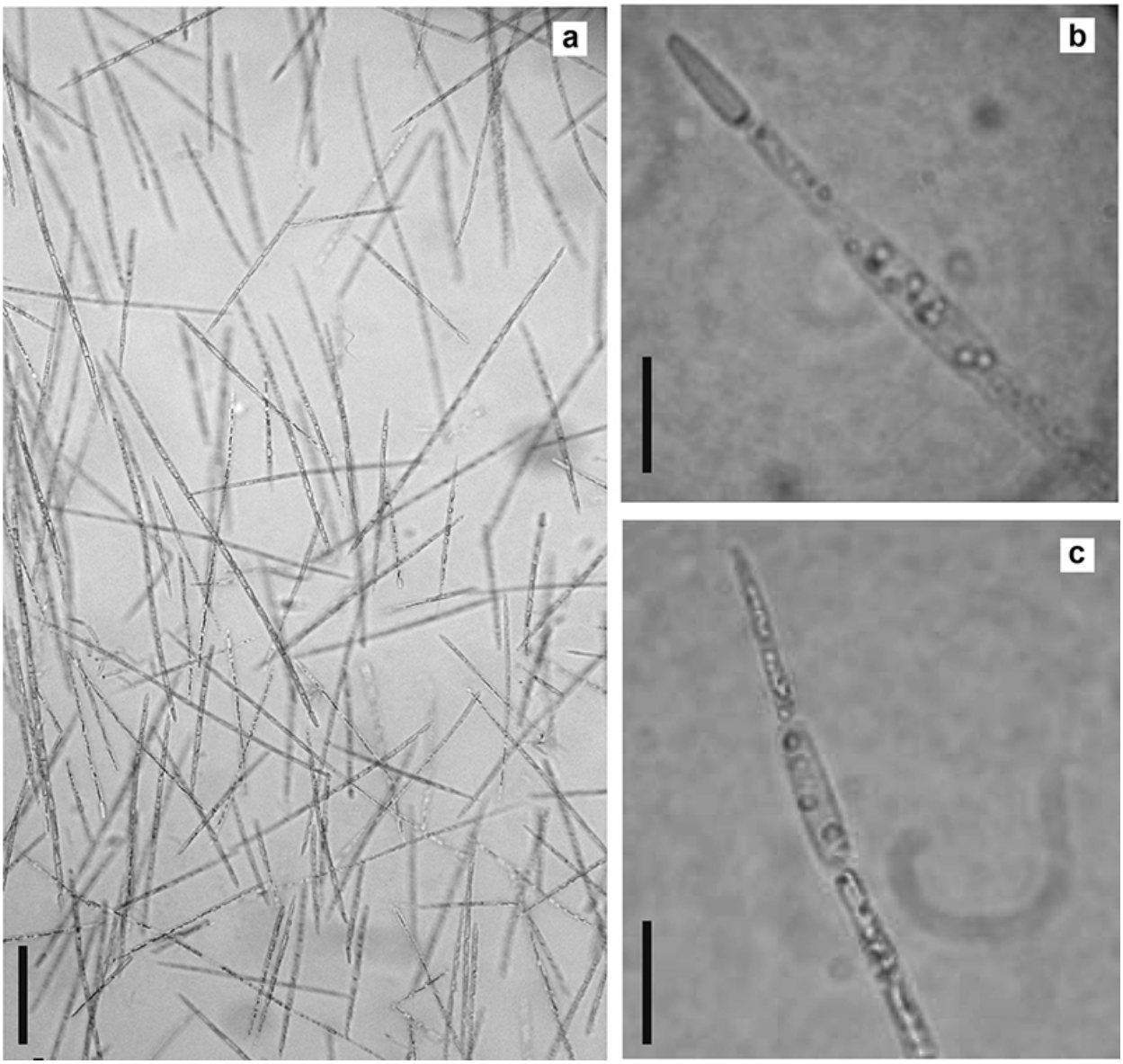


Fig. 4a-c. Photomicrographs of *Raphidiopsis raciborskii* bloom from the studied lake demonstrating: a. general aspect of the bloom; c. part of a trichome with a heterocyte and an akinete; c. part of a trichome with only an akinete. Bars =10µm.

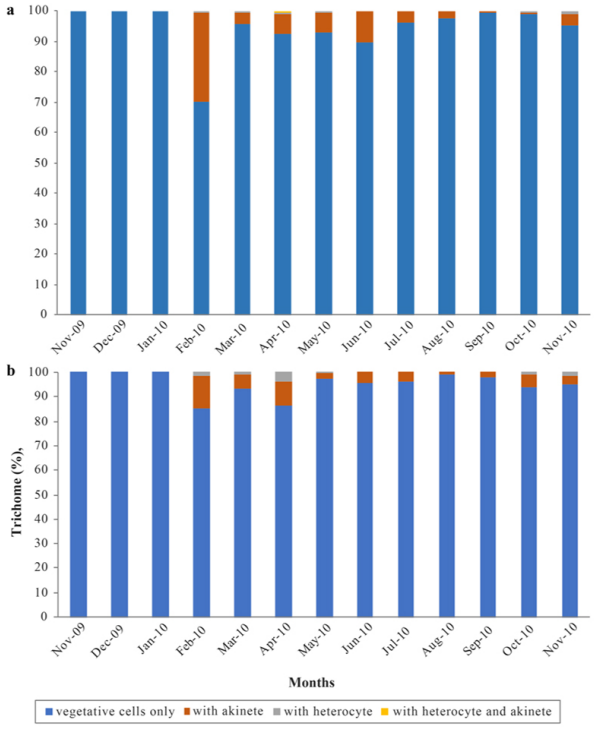


Fig. 5a, b. Bar graphs showing the percentage of *Raphidiopsis raciborskii* trichomes that are vegetative cells only, with akinetes, with heterocytes, or with both heterocytes and akinetes. Data represent samples taken from the lake inflow (a) and outflow (b), every month from November 2009 to November 2010.

We found high densities of *R. raciborskii*, including the formation of a yellow surface scum at low temperatures (12.6 - 15.5 ºC: June to August 2010), reaching 72,145 ind. mL^{-1} (12.6 °C), 72,285 ind.mL^{-1} (15.5 °C), and 89,964 ind. mL^{-1} and 130,475 ind.mL^{-1} (14.5 °C). During our study, the highest species density (199,550 ind.mL^{-1}) was at 26.6 ºC. Bloom occurrence at low temperatures is frequent in water bodies around the state of Rio Grande do Sul, and *R. raciborskii* is normally found throughout the year. In a subtropical lake (Lajeado, RS, 29º27'52.34"S and 51º58'16.03"W), the species reached 2,118 ind.mL^{-1} at 13.3 ºC, forming tiny flakes in the water (Hepp 2009). In a dam located in the mountains of the state of Rio Grande do Sul (29º13'30"S and 51º20'52"W), high densities of the species were recorded between 11 ºC and 24 ºC. However, the density when the water temperature was 11 ºC (0 ºC - air) was the highest found during the observed period (100,542 ind.mL^{-1}). On the other hand, on one occasion at 24 °C, the species only reached 8,211 ind.mL^{-1} and in another, 19 ind.mL^{-1} (A.T. Giordani - personal communication). These results, together with the observations by Dokulil & Mayer (1996), support the data by Komárková *et al.* (1999) that *R. raciborskii* has a wide tolerance to temperature, as long as the nutrients are appropriate and there are no competitors or predators.

Nitrogen availability in the lake was high throughout the study, especially ammonium [(60-)140-660 µg.L^{-1}], which is the preferred form of nitrogen for *R. raciborskii*. Throughout our study, ammonium ranged from (60)160-630 µg.L^{-1} (380 ± 150 µg.L^{-1}) – inflow and (60)140-660 µg.L^{-1} (379 ± 157 µg.L^{-1}) – outflow (Table 3). Since nitrogen was not limiting, there was no need for nitrogen fixation as a competitive strategy for *R. raciborskii* and, consequently, it was not necessary to use ATP for heterocyte formation. This justifies the low percentage (5.2%) of trichomes with heterocytes during the study. In general, low numbers of trichomes with heterocytes are common in *R. raciborskii* blooms and have been observed in other Brazilian waters by Branco & Senna (1994), Bouvy *et al.* (1999), Huszar *et al.* (2000) and Tucci & Sant'Anna (2003).

Phosphorus concentrations were low throughout the study, with orthophosphate values always less than 10 µg.L^{-1} (Table 3). However, these levels were not limiting for *R. raciborskii*, since the species has the capacity to store phosphorus and grow in concentrations that are limiting for other cyanobacteria (Jensen *et al.* 1994, Padisák 1997). This can explain the fact that blooms of this species followed mixed blooms of chroococcalean cyanobacteria (*Microcystis* spp.) found in the lake previously (V.R. Werner & H.D. Laughinghouse IV - personal observation), corroborating with observations by Tucci & Sant'Anna (2003) in blooms of Lake Garças (São Paulo, Brazil, 23°35'15.00"S and 46°39'38.12"W), where *Microcystis aeruginosa* (Kützing) Kützing was followed by *R. raciborskii*.

Table 2. Pearson correlation coefficients between abiotic variables and the first two axes of the PCA ordination (n=26).

	Principal components	
Variables	Axis 1	Axis 2
Temperature	-0.406	-0.769
Transparency	-0.843	0.227
pH	0.691	-0.642
Conductivity	0.872	0.062
Dissolved oxygen	0.625	-0.021
Ammonium	-0.728	0.006
Organic material	0.826	0.159
Total Explanation	51.8%	21.5%

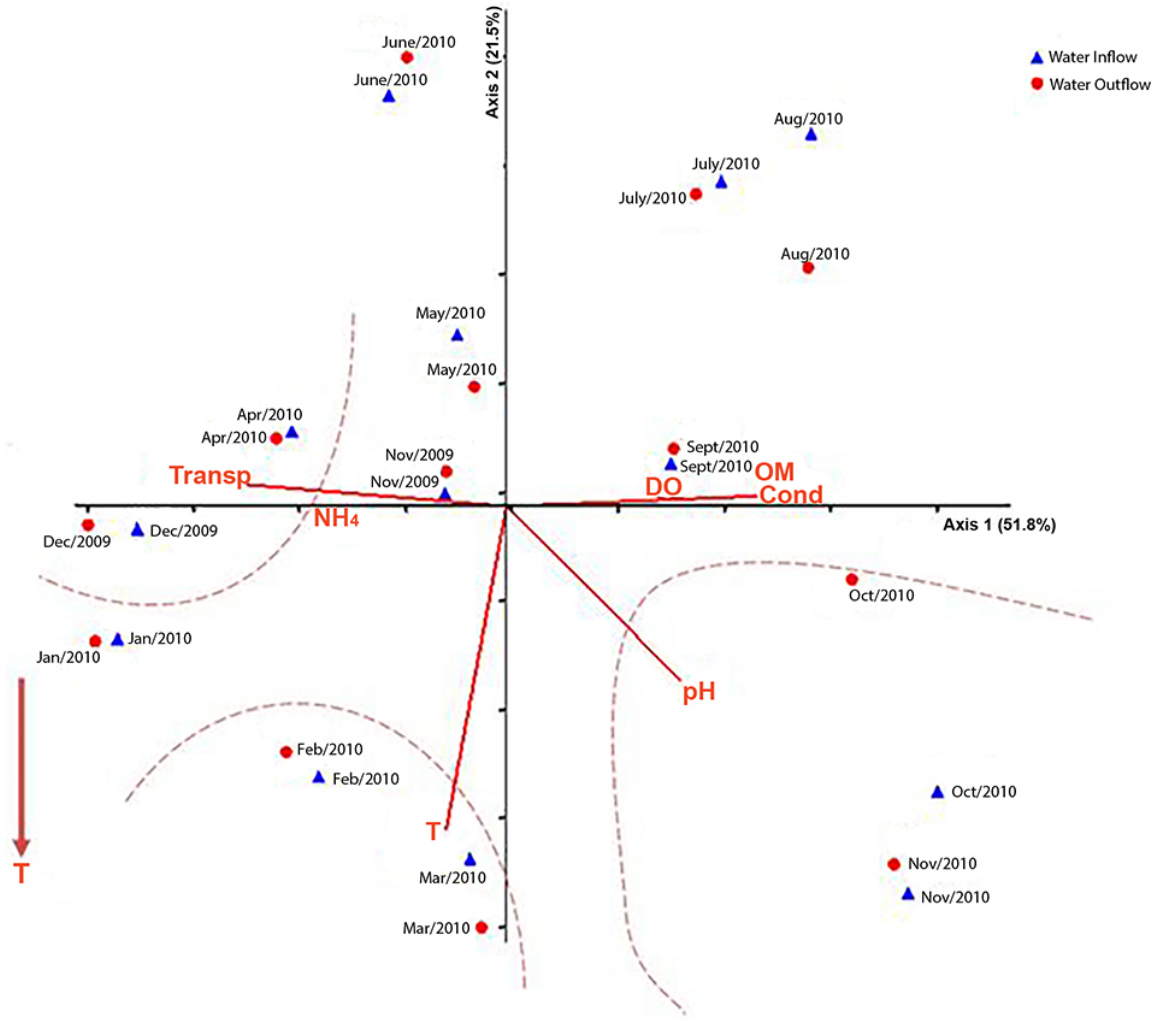


Fig. 6. Principal Component Analysis (PCA) ordination of the sampling units (P1) = water inflow and (P2) = water outflow, and of the physical and chemical variables analyzed. The sampling units are listed by month abbreviated as follows: Jan = January; Feb = February; Mar = March; Apr = April; May = May; Jun = June; Jul = July; Aug = August; Sept = September; Oct = October; Nov = November; Dec = December. T = Temperature; Transp = Transparency; Cond = Conductivity; DO = Dissolved oxygen; NH4 = Ammonium; OM = Organic material.

The organic matter in the lake ranged from 6.8-11.5 mg.L^{-1}, with the minimum value found at the end of spring (outflow: December 2009) and the maximum recorded at both collection sites in spring (October 2010). The mean electrical conductivity was 152.1 µS.cm^{-1}, with a minimum of 80.9 µS.cm^{-1} in summer (February 2010) and maximum of 273.4 µS.cm^{-1} in spring (October 2010), both at the inflow. Hence, the populations of *R. raciborskii* were able to tolerate large differences in ionic composition, which was also documented in the Ingazeira Reservoir (Pernambuco, Brazil) (Bouvy *et al.* 1999).

Researchers (*e.g.* Fogg *et al.* 1973, Pádisak 1997) have previously agreed that *R. raciborskii* develops in alkaline waters and does not occur in acidic waters. However, the pH of the water in our study varied from (5.4)5.6-8.7 and the densities of *R. raciborskii* were high, reaching 89,964 ind.mL^{-1} at pH 5.4; 72,285 ind.mL^{-1} and 99,994 ind.mL^{-1} at pH 5.6; and 61,400 ind.mL^{-1} at pH 8.7. In a study on the Pequeno River (23º33'52.16" S and 46º45'14.16"W - São Paulo, Brazil), a pH of 5.4 was recorded during a *R. raciborskii* bloom (Souza *et al.* 1998); however, the authors found that chlorophytes then became abundant, which did not occur in our study since *R. raciborskii* densities were persistently high. In Brazilian water bodies, high densities of *R. raciborskii* usually occur in alkaline waters, with pH varying between 8 and 9.4 (Bouvy *et al.* 1999, Huszar *et al.* 2000, Costa *et al.* 2006, Gemelgo *et al.* 2008). Nevertheless, blooms have also been recorded in slightly acidic to alkaline waters, with pH between 6 and 10 (Branco & Senna 1994, Komárková *et al.* 1999, Tucci & Sant'Anna 2003, Hepp 2009). These results, in addition to ours, indicate that *R. raciborskii* has a broad tolerance to pH.

Table 3. Abiotic variables recorded in the two samplings sites (water inflow and outflow) of the studied lake, from November 2009 to November 2010, with mean and standard deviation (Mean±SD). (ND = not detected, NM = not measured).

Site	Variables	Month													Mean±SD
		Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	
Inflow	Air temperature (°C)	NM	22.4	23.4	26	25.7	21.8	18.1	21.3	13.5	11.2	19	20.3	23.1	20.5±4.5
	Water temperature (°C)	22.8	23.4	27.2	28	26.6	23.5	19.4	15.5	13.3	13	19.4	24.2	24.3	21.6±5.1
	pH	6.3	6	6	6.7	7.8	5.9	6	5.6	6.7	6.5	6.7	8.2	8.7	6.7±1
	Ammonium (µg.L^{-1})	60	630	520	400	510	440	500	400	300	280	260	180	160	380±150
	Nitrate (µg.L^{-1})	ND	1,200	1,000	1,000	1,100	1,000	1,000	1,000	1,000	1,000	1,000	1,300	1,000	1,050±100
	Nitrite (µg.L^{-1})	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Orthophosphate (µg.L^{-1})	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Dissolved oxygen (mg.L^{-1})	6.40	6.50	4.00	6.70	8.20	7.20	9.00	2.50	9.80	9.00	9.00	8.30	7.80	7.30±2.10
	DO saturation (%)	73.2	75.8	46.6	85.1	99.2	84	97	24.8	93	83.5	97	99	92.6	80.8±22.2
	Organic material (mg.L^{-1})	10	7.2	8.5	9.5	9.7	9.8	9.8	10.2	10.2	10.8	10.9	11.2	10.6	9.9±1.1
	Conductivity (µS. cm^{-1})	94.8	92.8	92.9	80.9	119	129	147	165	189	239	122	273.4	227.8	151.7±62.9
	Transparency (m)	NM	0.35	0.40	0.30	0.25	0.45	0.30	0.35	0.25	0.22	0.15	0.15	0.10	0.27±0.11
	Depth (m)	NM	0.40	0.40	0.35	0.40	0.50	0.40	0.35	0.40	0.30	0.33	0.40	0.27	0.37±0.06
Outflow	Air temperature (°C)	NM	20	21.8	24.2	26	25.3	20.2	21	9.5	11.6	16.9	20.4	22	19.9±5
	Water temperature (°C)	22.7	23.3	27.7	28	26.2	24.5	18.9	14.5	14.5	12.6	19.8	22.2	25.8	21.6±5.2
	pH	6.3	6.1	5.9	6.5	8.4	5.6	6.6	5.4	6.4	7.7	6.7	7.5	8.2	6.7±1
	Ammonium (µg.L-1)	60	660	520	430	500	400	500	400	330	280	240	140	150	379±157
	Nitrate (µg.L-1)	ND	1,200	1,000	1,000	1,100	1,000	1,000	1,000	1,000	1,000	1,000	1,200	1,000	1,000±100
	Nitrite (µg.L-1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Orthophosphate (µg.L-1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Dissolved oxygen (mg.L-1)	7.70	6.60	3.20	6.00	8.00	7.50	8.20	3.60	10.50	8.30	9.00	8.10	8.00	7.28±2.03
	DO saturation (%)	88	77	36.6	76.2	98.6	89	88.4	35	97.1	99.7	99	97.6	98.6	83.1±22.5
	Organic material (mg .L-1)	10	6.8	8.5	9.5	8.8	9.8	9.8	10.2	10.2	10.5	11.4	11.5	10.8	9.8±1.3
	Conductivity (µS. cm-1)	100	91.7	94.9	81.5	128	122	143	142	187	207	130	264.6	259.3	150.1±61.3
	Transparency (m)	NM	0.40	0.40	0.30	0.25	0.45	0.30	0.30	0.25	0.23	0.20	0.27	0.15	0.29±0.09
	Depth (m)	NM	0.40	0.40	0.35	1.90	1.80	1.75	1.75	1.90	1.95	2.00	1.85	1.85	1.49±0.67

Toxicity

Raphidiopsis raciborskii can produce both saxitoxins (neurotoxin) that inhibit nerve conduction causing paralysis and respiratory failure, and cylindrospermopsins (hepatotoxin) that can cause necrosis of the liver, kidneys, lung and gastric mucosa (Falconer *et al.* 1994). Saxitoxin production from toxic strains of the species has already been reported for several Brazilian waters, with the following variants: saxitoxin, neosaxitoxin, dc-saxitoxin, dc-neosaxitoxin, and gonyautoxins GTX-1, GTX-2, GTX-3, GTX-4 and GTX-6 (Lagos *et al.* 1999, Bouvy *et al.* 1999, Yunes *et al.* 2003, Hepp 2009). Cylindrospermopsin has been documented in activated charcoal filter samples from a dialysis treatment in Caruaru, Pernambuco (Carmichael 2001, Marinho *et al.* 2005).

Saxitoxin (STX) and two gonyautoxin variants (GTX 2 and GTX 3) were present in the bloom (Table 4). The values were high when compared to the data available in the AGUAAN Program (Streamlining the Management and Use of Water with Harmful Algae) of the Cyanobacteria Research Unit of the Federal University of Rio Grande (FURG), Brazil, which has monitored surface waters in Rio Grande do Sul for ten years. In addition, our saxitoxin variants match the forms and quantities found in other studies throughout the State (Yunes *et al.* 2010). An important factor contributing to the toxic concentration of the bloom was the high density of *R. raciborskii* (> 1x10^6 cells.mL^{-1}).

Table 4. Cyanotoxins and *Raphidiopsis raciborskii* bloom densities (ind.mL^{-1}) from the studied lake (STX = saxitoxin, GTX = gonyautoxin) sampled at the sites in November 2010.

Sites		Water Inflow	Water Outflow
Bloom density (ind.mL^{-1})		83,504	61,400
Neurotoxins (µg.L^{-1})	STX	1.00	0.88
	GTX 2	3.50	3.06
	GTX 3	3.18	2.65
	STX (equivalents)	4.29	3.67

Blooms of *Raphidiopsis raciborskii* have been documented worldwide (as *Cylindrospermopsis raciborskii*) under different environmental conditions, from fresh to brackish waters, in tropical, subtropical and temperate zones, indicating that the species has a wide range of ecophysiological tolerances, corroborating the comments of Padisák (1997). This species is a more recent invader in temperate zones and its increase has been associated to climate change (*e.g.* Sinha *et al.* 2012). In addition to its adaptive strategies, the species is highly competitive in eutrophic environments, with the ability to form dense blooms and produce toxins. The ecological success of the species is related to: 1) migration in the water column; 2) tolerance to low light; 3) ability to use internal sources of phosphorus; 4) high absorption capacity of phosphorus and ammonia; 5) fixation of atmospheric nitrogen; 6) resistance to herbivory of zooplankton; 7) high dispersibility in rivers and especially in temperate lakes, via resistant akinetes, spread by birds and other agents; and 8) survival in slightly saline waters (Padisák 1997). The ammonium and phosphorus absorption capacity, as well as the ability to use internal sources of phosphorus, were key factors for bloom development of *R. raciborskii*. Furthermore, high competitiveness in eutrophic environments explains its excessive growth and consequent dominance, replacing mixed blooms formed by *Microcystis aeruginosa*, *M.* cf. *brasiliensis* (Azevedo & Sant'Anna) Rigonato *et al.*, *M. protocystis* Crow and *Radiocystis fernandoi* Komárek & Komárková-Legnerová that had been observed earlier (since 2005) in this water body - (V.R. Werner & H.D. Laughinghouse IV - personal observations).

The environmental conditions were favorable to maintain high densities of *R. raciborskii* in the lake throughout the study period. Due to the ability to produce toxins, these blooms are a permanent risk for aquatic biota, endangering animals that use lake water as a habitat, birds that use it as a resting and breeding area, and humans that use its surrounding area for recreational activities. Current studies on cyanobacterial blooms have proven their ability to produce aerosols containing cyanotoxins (Cheng *et al.* 2007, Stommel *et al.* 2013, Jang *et al*. 2020), a putative major human health threat for passersby and those that use contaminated lakes for leisure. These studies also indicate these aerosols as a risk factor for amyotrophic lateral sclerosis and cancer (Stommel *et al.* 2013, Zhang *et al.* 2015). As students and faculty are using this area for recreation, the possibility of exposure to both toxins and cyanobacteria through the air/wind is high, indicating the need for continued monitoring of this system and similar systems. This study provides insight into *R. raciborskii* bloom physiochemical ecology, which is appropriate for better management practices to mitigate harmful algal blooms.

ACKNOWLEDGEMENTS

To Research Support Foundation of the State of Rio Grande do Sul (FAPERGS) for the Scientific Initiation Grant to LM Silva (0786-2.03 / 09). To the Lutheran University of Brazil (ULBRA), *campus* Canoas, for permission to collect in the studied lake. To Manoel Luiz Nunes for the chemical analyses, Rosana Moreno Senna for identifying the trees and Ricardo Aranha Ramos for the map of the studied area. To Dr Fabiana Schneck for the valuable comments that improved the manuscript.

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