Permanent occurrence of *Raphidiopsis raciborskii* and cyanotoxins in a subtropical reservoir polluted by domestic effluents (Itupararanga reservoir, São Paulo, Brazil)

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Research Article

Abstract

Toxic cyanobacteria blooms are a frequent problem in subtropical reservoirs and freshwater systems. The purpose of this study was to investigate the occurrence of potentially toxic cyanobacteria and the environmental conditions associated with the presence of cyanotoxins in a Brazilian subtropical reservoir. Five collections were carried out at seven sampling locations in the reservoir, during the rainy and dry seasons, between the years 2016 and 2017. There was permanent occurrence of *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno, ranging between dominant and abundant, with an average biomass of 38.8 ± 29.9 mg L^{-1}. Also abundant were *D. solitarium*, *D. planctonicum*, *P. isothrix*, and *A. gracile.* Saxitoxin (STX) was detected in all the collected samples (0.11 ± 0.05 µg L^{-1}). Microcystin (MC) was also detected, but at lower concentrations (0.01 ± 0.0 µg L^{-1}). Low availability of NO_3^- and phosphorus limitation had significant effects on the *R. raciborskii* biomass and the levels of STX and MC. It was observed that *R. raciborskii* was sensitive to thermal stratification, at the same time that STX levels were higher. This suggested that STX was produced under conditions that restricted the growth of *R. raciborskii*. These are important findings, because they add information about the permanent occurrence of STX and *R. raciborskii* in an aquatic ecosystem limited by phosphorus, vulnerable to climatic variations, and polluted by domestic effluents.

Introduction

Tropical and subtropical regions are those most affected by the occurrence of potentially toxic cyanobacteria blooms (WHO 2017). Freshwater ecosystems in these regions are environments conducive to the occurrence of cyanobacteria and cyanotoxins, because in addition to the favorable climate, they frequently contain high levels of nutrients (Mowe et al. 2015).

Brazil was the first country to create specific legislation containing guidelines aimed at controlling cyanotoxins and cyanobacteria in drinking water (Bittencourt-Oliveira et al. 2014). This occurred after the reported intoxication of 131 chronic kidney disease patients at a hemodialysis clinic in the city of Caruarú (Pernambuco State, Brazil), due to contamination of the water by microcystins (Carmichael et al. 2001). Studies have reported the occurrence of blooms of potentially toxic species in different regions of Brazil, mainly associated with the presence of *R. raciborskii* and *Microcystis* spp (Figueredo & Giani 2009; Moschini-Carlos et al. 2009; Cunha & Calijuri 2011b; Bittencourt-Oliveira et al. 2014; Fonseca et al. 2014; Nishimura et al. 2014; Machado et al. 2016; Casali et al. 2017; Santos et al. 2018; Vicentin et al. 2018; Rodrigues et al. 2019). The identified cyanotoxins included saxitoxins, microcystins, and cylindrospermopsin (Molica et al. 2002; Moschini-Carlos et al. 2009; Bittencourt-Oliveira et al. 2014; Casali et al. 2017; Pedrosa et al. 2020).

Toxic cyanobacteria blooms in eutrophic reservoirs represent a major public health problem. Pedrosa et al. (2020) reported that saxitoxin (STX) had a synergistic effect in Zika virus infections in Northeast Brazil, even at concentrations considered safe (<3.00 µg L^{-1}). Facciponte et al. (2018) investigated the exposure route of aerosolized STX, showing contamination of the human respiratory tract. Christensen

and Khan (2020) reported several other routes of exposure to this toxin, leading to lethal and sublethal effects, together with bioaccumulation, due to different types of direct and indirect contact.

The occurrence of microcystins (MC) has been associated with high concentrations of nitrogen, with blooms generally occurring during the rainy season (Mowe et al. 2015). Worldwide, the taxa frequently identified in blooms with the presence of MC are *Microcystis aeruginosa*, *M. protocystis*, *M. panniformis*, *Plantothrix agardhii*, *P. isothrix*, and *Dolichospermum* (Molica et al. 2002; Moschini-Carlos et al. 2009; Sant'Anna et al. 2011; Paerl & Paul, 2012; Merel et al. 2013; Bittencourt-Oliveira et al. 2014; Funari et al. 2017; Huisman et al. 2018; Pham et al. 2020). Blooms dominated by these species are more frequent in the summer, when the warm climate favors an increase of biomass and the occurrence of cyanotoxins tends to be higher (Trung et al. 2018). Although the levels of cyanotoxins are not strongly correlated with biomass increase, the production of MC can be stimulated in the presence of some chlorophytes (Bittencourt-Oliveira et al. 2015). In addition to allelopathic interactions, herbicides used in agriculture can be carried to aquatic ecosystems and favor the production of cyanotoxins (Brêda-Alves et al. 2020).

The occurrence of STX, produced by toxic strains of *R. raciborskii*, is related to high temperatures, being common in periods of drought and low nitrogen availability (Mowe et al. 2015). The ability to fix atmospheric nitrogen is one of the main adaptations of *R. raciborskii*, enabling its growth in environments limited by phosphorus (Kenesi et al. 2009; Piccini et al. 2011). This allows the maintenance of these species in different environments, despite not necessarily being correlated to environmental variables (Figueredo & Giani 2009). In addition, some studies mention the allelopathic effect of *R. raciborskii*, inhibiting the development of species of the genus *Microcystis*, favoring monospecific blooms (Mello et al. 2012). Furthermore, its sensitivity is related to stratification of the water column and flushing (Reynolds et al., 2002; Padisák et al., 2009). However, the many ecophysiological adaptations reflect the high genetic variability of this species, accounting for its wide occurrence in different regions of the world (Piccini et al. 2011; Antunes et al. 2015; Vanderley et al. 2021).

The problem of eutrophication is global, with widespread occurrence of potentially toxic species, highlighting the need to understand the most important environmental factors influencing this phenomenon (Moschini-Carlos et al. 2009, 2010; Yamamoto et al. 2011; Paerl & Paul, 2012; Merel et al. 2013; Beghelli et al. 2016; Machado et al. 2016; Huisman et al. 2018; Pompêo et al. 2021). Such studies are crucial to support strategies for the management of these environments, aiming at achieving good ecological status and guiding public health actions (Cardoso-Silva et al. 2015; Pompêo et al. 2021). Therefore, in order to better understand the dynamics of cyanobacteria in an eutrophic subtropical reservoir, the aims of this study were to i) investigate the occurrence of cyanotoxins (microcystin and saxitoxin), ii) elucidate the relationship between cyanotoxins and the cyanobacteria biomass, and iii) identify *in situ* the environmental conditions that significantly influence the presence of cyanotoxins.

Materials And Methods

Study area

The Itupararanga reservoir is located in the southeastern region of São Paulo State, Brazil (Fig. 1). The main channel of the reservoir is 26 km long and the average depth is 7.8 m, reaching 21 m. The climate in the region is typically subtropical and the average temperature varies between 18 and 22 °C (Melo et al., 2019).

Approximately 63% of the water of this reservoir is used for public supply in several cities located in its vicinity, serving around 800,000 people (Pedrazzi et al. 2013; Rosa et al. 2015). Recent studies have reported significant degradation of the reservoir water quality, due to inflows of domestic effluents in the fluvial region (Cunha & Calijuri 2011a; Taniwaki et al. 2013; Beghelli et al. 2016; Melo et al. 2019). During the period of sample collection, the reservoir was characterized as super-eutrophic (Melo et al. 2019), based on the trophic status index model adapted by Cunha et al. (2013) for tropical and subtropical reservoirs.

Samples were collected at seven points (P1-P7) along the longitudinal axis of the reservoir (Fig. 1). The choice of locations considered the characteristics of use and occupation of the margins, as described by Rosa et al. (2015). Five collections were performed during the rainy and dry seasons, in the years 2016 and 2017 (December 2016, March 2017, August 2017, October 2017, and December 2017). The rainy season was from October to March and the dry season was from April to September (Melo et al. 2019).

Data reported previously for the Itupararanga reservoir (Melo et al. 2019) were used as a reference for the limnological characterization of the water, considering the following parameters: total nitrogen (TN), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), total phosphorus (TP), orthophosphate (PO_4^{3-}), chlorophyll-a (Chl-a), euphotic zone (EZ), depth, temperature (T), pH, electrical conductivity (EC), turbidity (TURB), and redox potential (Eh).

For quantitative analysis of phytoplankton, integrated samples of the water column were collected with a plastic hose (1 m length) and were fixed with 1% acetic lugol. The identification of cyanobacteria was based on the studies of Komárek & Anagnostidis (1999, 2005), Sant'Anna et al. (2004, 2006, 2012), and Nogueira et al. (2011). Counting was performed as described by Utermöhl (1958), using an inverted Zeiss AxioVert microscope. The biovolume was determined as described by Hillebrand et al. (1999) and Sun & Liu (2003). Phytoplankton biomass was estimated according to Wetzel and Likens (2000), where 1 mm^3 L^{-1} = 1 mg L^{-1}. Species were considered abundant when their biomass represented more than 5% of the total biomass.

Samples were collected for analysis of free STX and MC present in the reservoir water. In the laboratory, the samples were filtered using glass fiber filters (Whatman GFC) with pore size of 1.2 μm. The STX content was determined by enzyme-linked immunosorbent assay (ELISA), using the Saxitoxin Plate Kit (Beacon Analytical Systems, USA), according to the manufacturer's recommendations.

The analysis of microcystins was performed by liquid chromatography coupled with mass spectrometry (LC-MS). For this, a 400 mL volume of each sample was submitted to solid phase extraction (SPE) using 500 mg Sep-Pak C18 6cc Vac cartridges (Waters, USA), according to the methodology described by Kim

et al. (2009). The cartridges were previously conditioned with 10 mL of HPLC grade methanol, followed by 10 mL of Milli-Q water. After eluting the samples, the cartridges were cleaned with 10 mL of Milli-Q water and 10 mL of 20% methanol. Finally, the analytes of interest were eluted with 10 mL of 80% methanol and the eluate was dried at 35 ºC, under nitrogen. The dry material was resuspended in 1 mL of 70% methanol and filtered through a 0.45 µm PVDF membrane (Millipore, USA). The analysis employed a 1260 Infinity chromatograph (Agilent Technologies, USA), which was coupled to a triple quadrupole mass spectrometer (6460 Triple Quad LC/MS, Agilent Technologies, USA) equipped with electrospray ionization (ESI) and operated in positive mode at 3500 V (The Supplementary Information (IS) Table 1-2 and Fig. 1-2 supports this analysis).

The inclusion and exclusion criteria for the environmental variables used in the statistical analysis were based on the results obtained using the Pearson linear correlation test. The entire matrix of the set of physical, chemical, and biological water data was used, including cyanobacteria identified with abundance >5%. The classification of the magnitude of the coefficients was based on Cohen (1988). Next, canonical correspondence analysis (CCA) was used to identify the environmental variables and species correlated to STX and MC. For this, the data set was treated by log10 transformation. In order to identify the environmental variables that had a significant effect in the CCA model, the scores were subjected to analysis of variance (ANOVA, $p < 0.05$). A simple linear regression analysis was then performed, according to the patterns observed in the fitted CCA model, in order to determine the effects of the most significant environmental variables on the occurrence of cyanotoxins. All the analyses were performed using the R$^®$ 3.4.0 Vegan 2.5-2 software package (Oksanen et al. 2018).

Results

The Cyanophyceae were major contributors to the biomass of the phytoplankton community in the Itupararanga reservoir. Among all groups, cyanobacteria represented 40.3% of the total biomass, with higher levels at the end of the dry period (August 2017) and the beginning of the rainy period (October 2017) (Fig. 2). Among the abundant species identified, *R. raciborskii* was dominant in December 2016 and March 2017, with its occurrence remaining constant in the other collections (Fig. 3, Table 1). *R. raciborskii* showed permanent occurrence in the reservoir, with no significant seasonal variations of its biomass (mean 38.8 ± 29.9 mg L^{-1}). The apparently seasonal species included *Phormidium aerugineo-caeruleum* (Gomont) Anagnostidis & Komárek (mean 73.5 ± 89.6 mg L^{-1}), which only occurred at points P6 and P7, in August 2017. Other abundant species were *Dolichospermum solitarium* (Klebahn) Wacklin, L. Hoffmann & Komárek (mean 41.3 ± 73.5 mg L^{-1}), *Dolichospermum planctonicum* (Brunnthaler) Wacklin, L. Hoffmann & Komárek (mean 36.5 ± 60.3 mg L^{-1}), *Planktothrix isothrix* (Skuja) Komárek & Komárková (mean 25.8 ± 29.3 mg L^{-1}), and *Aphanizomenon gracile* Lemmermann (mean 14.7 ± 24 mg L^{-1}) (Fig. 3, Table 1).

Between the dry period (August 2017) and the beginning of the rainy period (October 2017), the cyanobacteria composition altered, with abundance of the species *D. solitarium*, *P. isothrix*, and *A. gracile*,

in addition to *R. raciborskii* (Fig. 3).

The average cyanobacteria biomass varied among the sampling periods, indicating a seasonal response of these organisms (Fig. 4a). Spatial heterogeneity of the reservoir was also evident, since the average cyanobacteria biomass varied among the sampling points (Fig. 4a).

Free STX was detected, with an average concentration of 0.11 ± 0.05 µg L^{-1} over the five collections. Among the MC variants evaluated, only MC-LR was detected at low concentrations (0.01 ± 0.0 µg L^{-1}) (Table 1).

Although both cyanotoxins were detected at low concentrations, the patterns of occurrence were different (Fig. 4b, Table 1). There was permanent presence of STX and MC-LR, with the compounds occurring in all five collections between December 2016 and December 2017. The lowest levels of STX were observed in August 2017, when the biomass values were also lower (Fig. 4b).

The environmental variables that showed significant effects in the CCA were TN, NO_2^-, NO_3^- and PO_4^{3-} (Table 1). The model provided high explanatory power, with the first two axes explaining 79.5% of the data variation (42.06% by axis 1 and 37.44% by axis 2) (Fig. 5).

Variables	avg.	min.	max.	SD
TN (µg L^{-1})	484,66	166,0	1210,00	215,16
NO_2- (µg L^{-1})	2,44	0,40	10,80	2,36
NO_3- (µg L^{-1})	81,09	7,90	295,80	83,22
PO_4^{3}- (µg L^{-1})	22,50	3,80	60,40	18,66
STX (µg L^{-1})	0,11	0,04	0,21	0,05
MC-LR (µg L^{-1})	0,01	0,00	0,02	0,00
Aphanizomenon gracile (mg L^{-1})	14,70	0,13	80,81	24,06
Raphidiopsis raciborskii (mg L^{-1})	38,80	3,43	119,76	29,99
Dolichospermum planctonicum (mg L^{-1})	36,54	3,00	143,22	60,37
Dolichospermum solitarium (mg L^{-1})	41,32	0,31	290,57	73,56
Phormidium aerugineo - caeruleum (mg L^{-1})	73,57	2,75	174,36	89,64
Planktothrix isothrix (mg L^{-1})	25,81	1,35	100,30	29,37

Table 1 Average (avg), minimum (min), maximum (max), and standard deviation (SD) values for the variables analyzed using CCA: total nitrogen (TN), nitrite (NO_2^-), nitrate (NO_3^-), and orthophosphate (PO_4^{3-}) (data reported by Melo et al. (2019) for the period from December 2016 to December 2017), and saxitoxin (STX), microcystin (MC-LR), and abundant cyanobacteria biomass (data obtained in the present study)

Only *R. raciborskii* was positively correlated with STX and MC-LR, ordered in the quadrant opposite the NO_3^- gradient. Among the species analyzed, no other organism showed a positive correlation with the occurrence of cyanotoxins. Linear regression analysis applied to the *R. raciborskii* biomass and STX values ($R^2 = 0.21$; $p < 0.05$) confirmed that the presence of STX in the reservoir depended on the biomass of this species (Fig. 6a). In addition, there was a statistically significant inverse linear relation between NO_3^- and *R. raciborskii* biomass ($R^2 = 0.41$; $p < 0.05$) (Fig. 6b). A negative correlation between STX and NO_3^- was observed in the Pearson correlation analysis ($r = -0.46$) (Table S3) and the CCA (Fig. 5), confirming that NO_3^- directly affected the occurrence of *R. raciborskii* and, consequently, the presence of STX in the reservoir (the Supplementary Information (IS) Table 3-4 supports this results).

In the case of MC-LR, despite transforming the values using log10, the normality test (W) indicated that the normality criterion was not met, so the linear effects of the variables on this toxin were not evaluated.

Discussion

The observed predominance of cyanobacteria in the Itupararanga reservoir was in agreement with previous findings (Cunha & Calijuri 2011b; Casali et al. 2017), where the presence of *R. raciborskii* was identified in the reservoir, its main tributaries, and areas close to the dam. However, Casali et al. (2017) identified an opposite pattern in the dominance of *R. raciborskii* with respect to the climate, with high biomass values in the dry period (>1.0 x 10^7 μm^3 mL^{-1}) and lower values in the rainy season (~5.0 x 10^6 μm^3 mL^{-1}). Other studies have also reported the abundance of *R. raciborskii* in Brazilian reservoirs in the dry and rainy seasons, in addition to species of the genera *Dolichospermum* and *Aphanizomenon* (Figueredo & Giani 2009; Moschini-Carlos et al. 2009; Sant'Anna et al. 2011; Bittencourt-Oliveira et al. 2014; Fonseca et al. 2014; Nishimura et al. 2014; Machado et al. 2016; Santos et al. 2018; Vicentin et al. 2018; Rodrigues et al. 2019).

The variation of the average cyanobacteria biomass among the samples revealed the influence of climatic conditions on these organisms. According to Melo et al. (2019), thermal stratification from point P4 to P7 was observed in October 2017, together with a substantial difference in levels of dissolved oxygen (DO) between the surface (7 mg L^{-1}) and the bottom (3 mg L^{-1}). In the same period, a decrease of the cyanobacteria biomass was observed at the sampling points where there was thermal stratification. According to Visser et al. (2016), thermal stratification tends to favor floating cyanobacteria, due to the weak mixing, allowing better access to light. In the present study, although the cyanobacteria biomass decreased at points where thermal stratification occurred, *R. raciborskii* and other species that also have

aerotopes (*D. solitarium*, *P. isothrix*, and *A. gracile*) were abundant. Furthermore, changes related to the availability of light, due to reduced water transparency, can have a significant influence on regulation of the dominance of cyanobacteria genera throughout the year (Vanderley et al. 2021).

The levels of STX and MC-LR identified were within the limits recommended by Brazilian legislation (Brazil, 2004) and the World Health Organization, of 3 µg L^{-1} for STX and 1 µg L^{-1} for MC (Chorus & Bartram 1999). Casali et al. (2017) investigated the presence of STX as a function of the density of *R. raciborskii* in the Itupararanga reservoir, obtaining values between 0.04 and 0.20 µg L^{-1}, which were close to those observed here, as well as strong correlation (r = 0.73, p <0.001) between the biomass and STX values. In the present study, although significant linear correlation was observed between *R. raciborskii* biomass and STX, the effect was classified as moderate (R^2 = 0.21; p <0.05).

The moderate linear relationship between the STX and biomass values could have been related to restrictive environmental conditions, which inhibited increase of the cyanobacteria volume and stimulated the production of cyanotoxins (Yamamoto et al. 2011; Lopes et al. 2012; Sarkar et al. 2020). For example, the phosphorus deficiency in the environment could have influenced the production of STX. Melo et al. (2019) observed a continuous decrease in the trophic status index towards the dam, influenced by phosphorus deficiency, classifying this region as meso-oligotrophic. Vargas (2012) tested different strains of *R. raciborskii* from the Itupararanga reservoir, finding that the production of STX by this species increased in an oligotrophic environment. These previous results corroborated the present findings, since higher levels of STX were observed in the region close to the Itupararanga reservoir dam (points P6 and P7).

It has been reported that environmental factors such as light, temperature, and nutrients have an important influence on the production of cyanotoxins, and that the consequences may be aggravated by climatic changes (Piccini et al. 2011; Paerl & Paul 2012; Antunes et al. 2015; Huisman et al. 2018; Pham et al. 2020,Vanderley et al. 2021). Melo et al. (2019) identified high concentrations of TN (484.66 ± 215.16 µg L^{-1}) in the Itupararanga reservoir during the same period as this study, reporting that the primary productivity of the reservoir was limited by phosphorus. The limitation by phosphorus in this reservoir has been reported in the last five years, prior to which there was co-limitation by phosphorus and nitrogen (Cunha & Calijuri 2011a, 2011b; Taniwaki et al. 2013; Beghelli et al. 2016; Casali et al. 2017; Melo et al. 2019).

The inverse correlation between NO_3^- and the occurrence of *R. raciborskii* and STX may have been related to the energy expenditure required for these organisms to fix inorganic forms of nitrogen. In the case of diazotrophic cyanobacteria, such as *R. raciborskii*, this occurs in environments limited by phosphorus (Kenesi et al. 2009). According to Brentano et al. (2016), STX biosynthesis is regulated by stress situations related to the depletion of inorganic nitrogen in the environment, restricting biomass increase. The release of STX then has the function of contributing to homeostasis of the organism, regulating cell permeability (Brentano et al. 2016; Brêda-Alves et al. 2020; Sarkar et al. 2020).

In the case of MC-LR, which was only detected at low concentrations, the maximum levels occurred in December 2016 and December 2017, considered rainy periods (Melo et al., 2019). At the same time, during these periods there was no abundance of species belonging to the genus *Microcystis*, but there was abundance of *P. isothrix*, *Dolichospermum* spp., and *P. aerugineo-caeruleum*. It is likely that the presence of MC in these periods was related to the occurrence of these organisms, since they are also potential producers of microcystins (Huisman et al. 2018).

In a study undertaken at the Billings complex reservoir and the Guarapiranga system in the metropolitan region of São Paulo, Moschini-Carlos et al. (2009) found moderate MC-LR concentrations of 0.28 µg L^{-1} in the rainy season and 0.57 µg L^{-1} in the dry season, with *P. agardhii* and *Microcystis* spp. being dominant. Bittencourt-Oliveira et al. (2014) found this toxin in 100% of the reservoirs studied in the Northeast region of Brazil, with high levels of MC ascribed to high biomasses of cyanobacteria of the genera *P. agardhii*, *P. isothrix*, and *Microcystis* spp..

Conclusions

The high contribution of Cyanophyceae in the Itupararanga reservoir was mainly due to the abundance of *R. raciborskii*. This species was the only one that had constant occurrence, being found in all the samples. The presence of STX was moderately influenced by *R. raciborskii* biomass, according to the classification parameters adopted. In this reservoir, a set of specific environmental conditions determined the variations of *R. raciborskii* biomass and STX. The facilitating environmental conditions included low availability of NO$_3^-$ and phosphorus limitation. The trophic state could have been an additional factor, since the STX levels followed an increasing trend in the region close to the dam, characterized as meso-oligotrophic. However, *R. raciborskii* was sensitive to thermal stratification, at the same time that STX levels increased. Hence, it appeared that STX was produced under conditions restrictive for the growth of *R. raciborskii*. These are important findings, since they provide information about the permanent occurrence of STX and *R. raciborskii* in an aquatic ecosystem with decrease of the trophic status index (upstream → downstream). Potentially toxic cyanobacteria had permanent abundance in the reservoir, with the alternation among them, due to changes in environmental conditions, implying the constant occurrence of cyanotoxins.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Competing interests: The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Data reported previously for the Itupararanga reservoir (Melo et al. 2019) were used as a reference for the limnological characterization of the water, available at: https://doi.org/10.1029/2019WR025991

Authors' contributions

All authors contributed to the design of this study, as follows: Leila Machado: writing original draft, formal analysis; Fabiane Dörr: chromatographic analysis, formal analysis; Felipe Dörr: chromatographic analysis, formal analysis; Daniele Frascareli: sample collections, formal analysis; Darllene Melo: sample collections, formal analysis; Erik Gontijo: sample collections, formal analysis; Kurt Friese: formal analysis, funding acquisition; André Rosa: formal analysis, funding acquisition; Ernani Pinto: formal analysis, chromatographic analysis; Marcelo Pompêo: formal analysis, funding acquisition; Viviane Moschini-Carlos: formal analysis, funding acquisition. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

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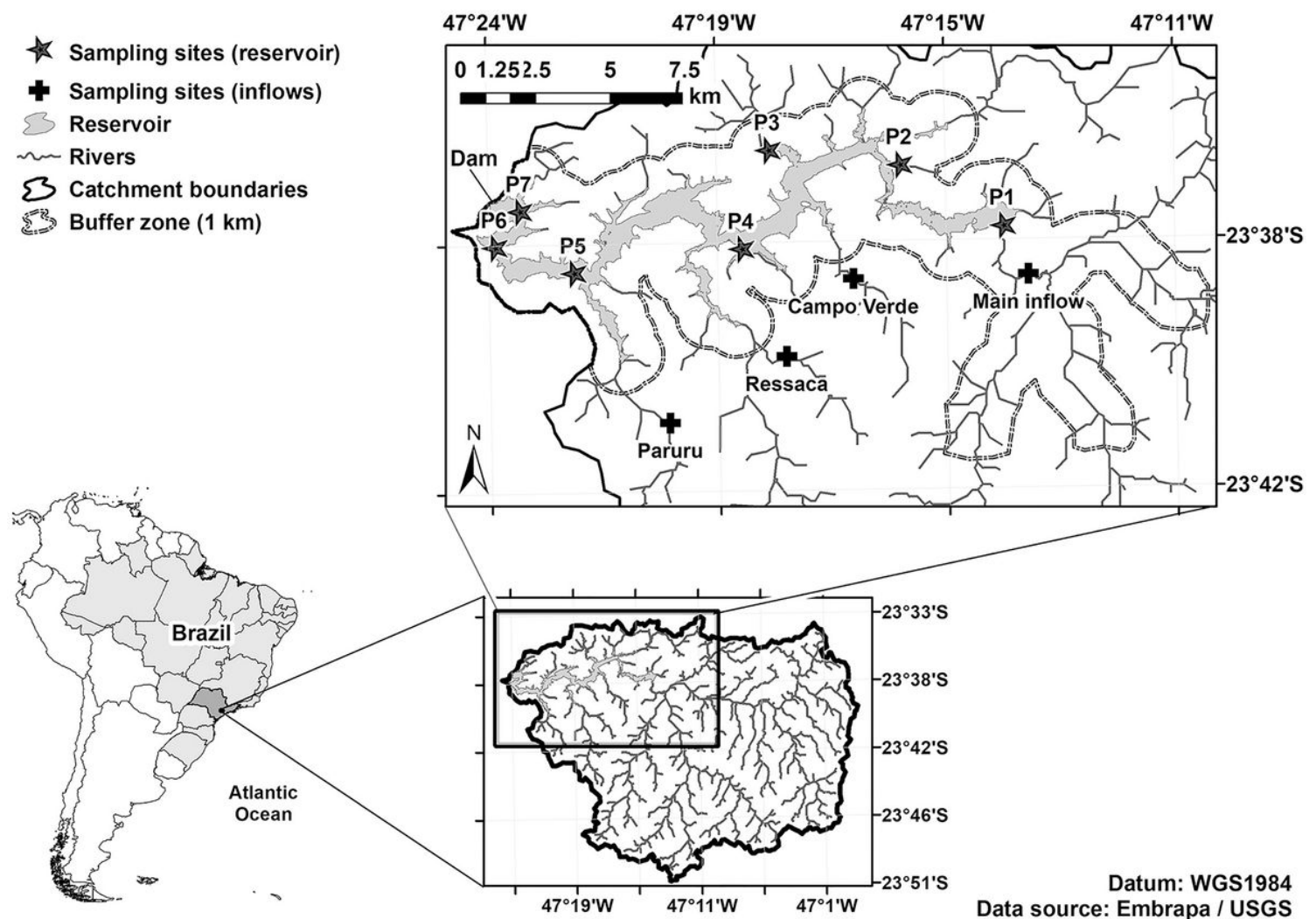
Figures

Figure 1

Map of the Itupararanga reservoir and locations of the sampling points. Original source: Melo et al. (2019)
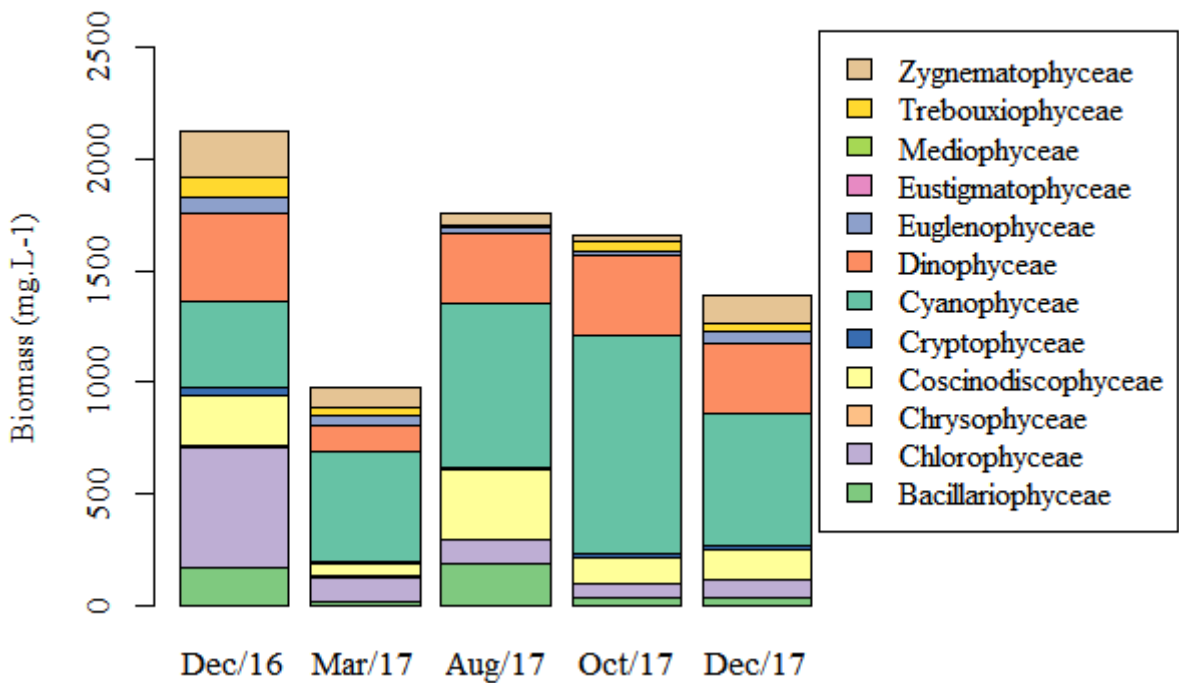

Figure 2

Total biomasses of the phytoplankton classes in the Itupararanga reservoir during the periods December 2016, March 2017, August 2017, October 2017, and December 2017
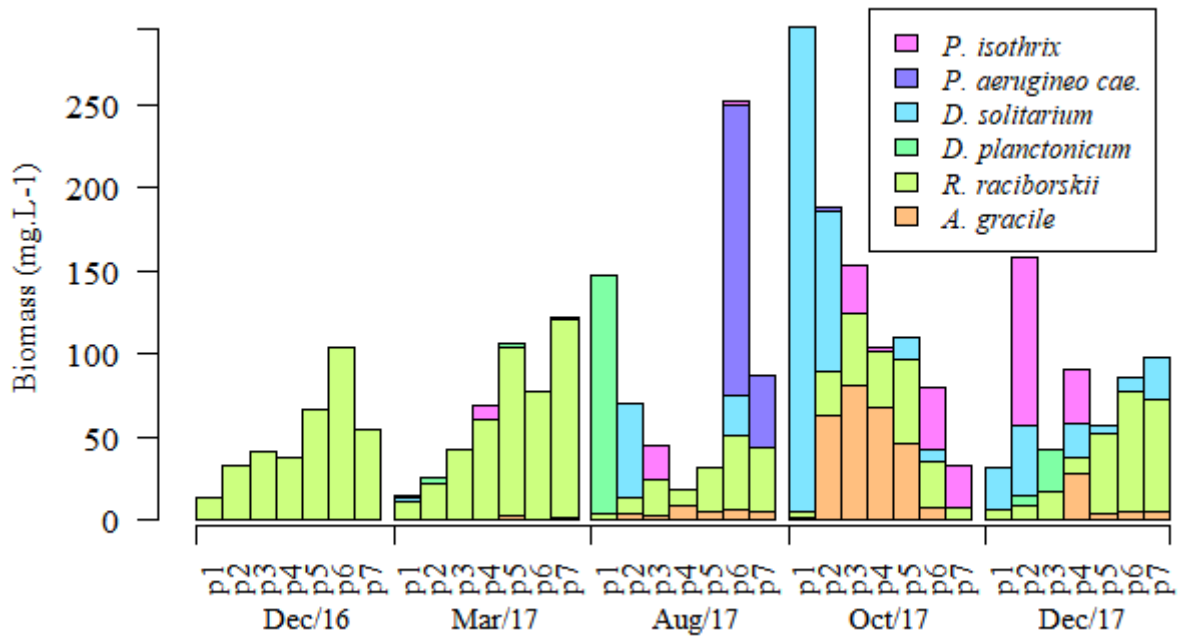
Figure 3

Biomasses of abundant cyanobacteria (>5%) during the periods December 2016, March 2017, August 2017, October 2017, and December 2017
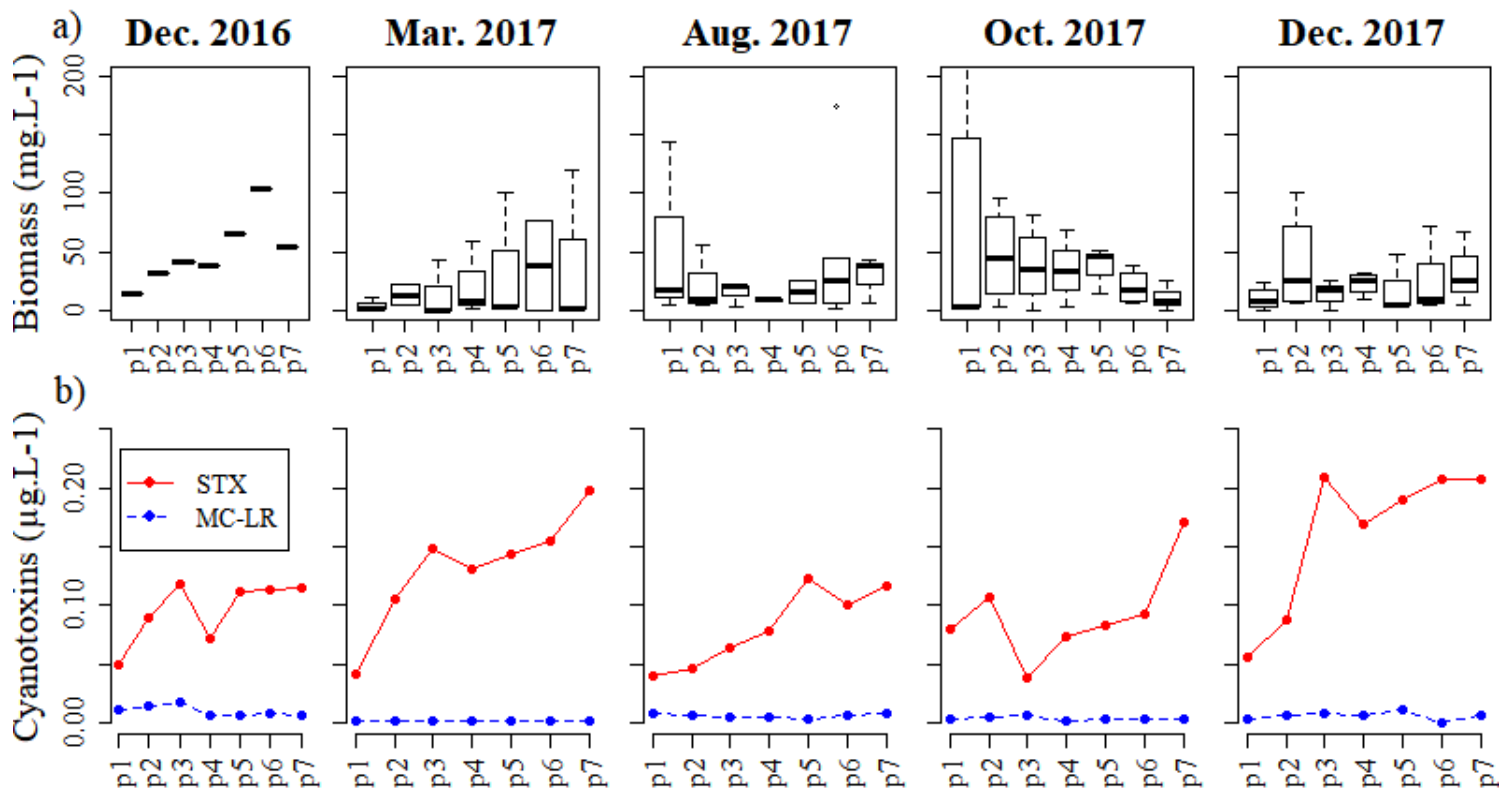

Figure 4

a) Box plots of cyanobacteria biomasses (mg L-1) in the different collections, and b) contents (µg L-1) of saxitoxin (STX) and microcystin (MC-LR)
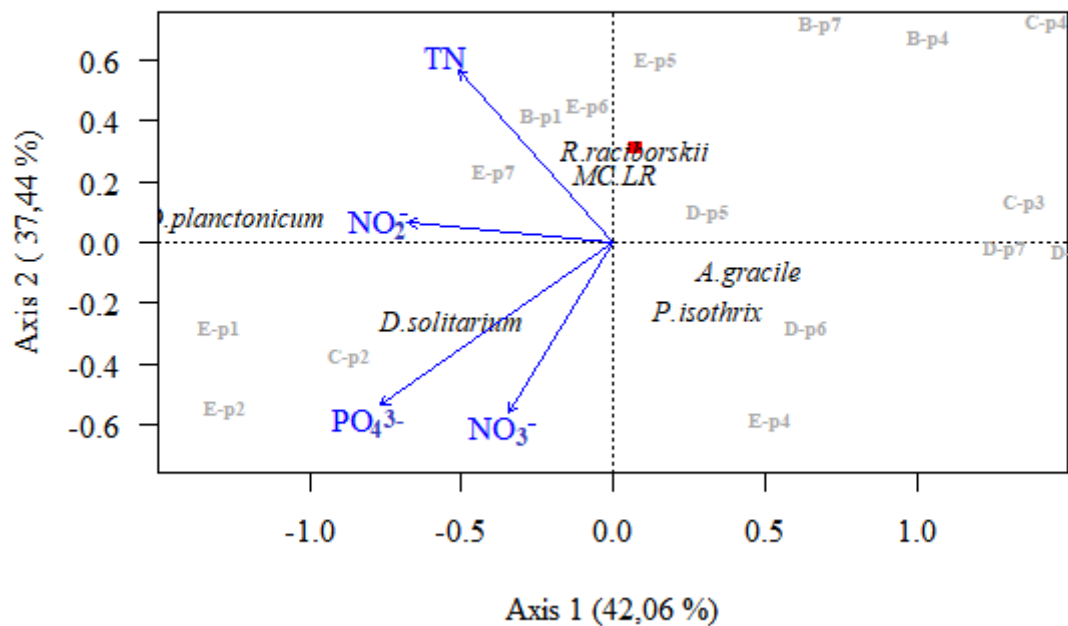

Figure 5

CCA biplot with the following variables: total nitrogen (TN), nitrite (NO2-), nitrate (NO3-), orthophosphate (PO43-), microcystin (MC-LR), saxitoxin (STX, represented by the symbol "■" in red), and abundant cyanobacteria biomass
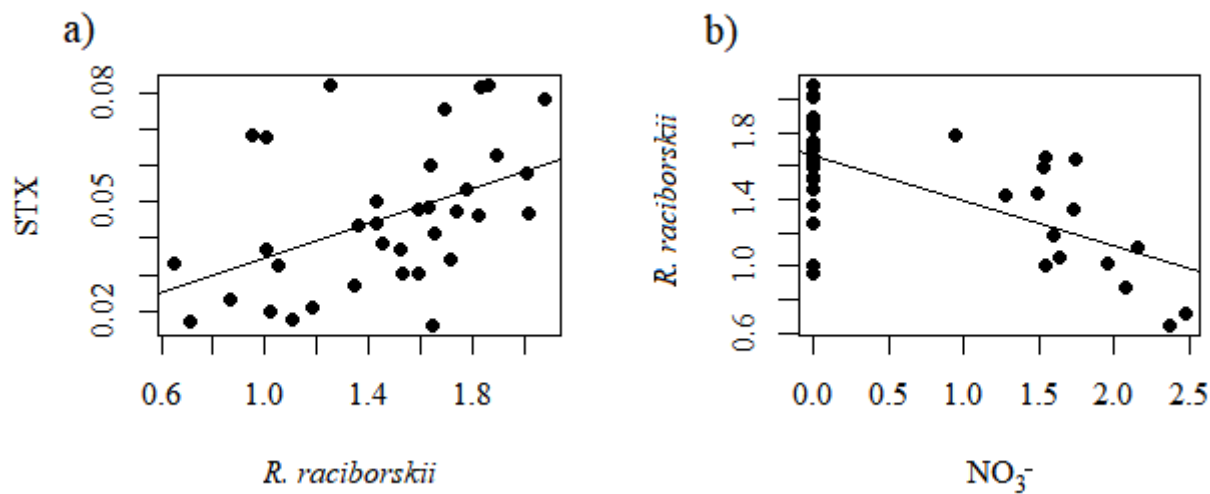

Figure 6

Simple linear regression between (a) R. raciborskii and STX (R2 = 0.21; p <0.05), and (b) NO3- and R. raciborskii (R2 = 0.41; p <0.05)

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