Journal of Applied Phycology (2020) 32:421–430
https://doi.org/10.1007/s10811-019-01972-w

Effect of nutrient concentration on growth and saxitoxin production of *Raphidiopsis raciborskii* (Cyanophyta) interacting with *Monoraphidium contortum* (Chlorophyceae)

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Received: 24 January 2019 / Revised and accepted: 22 October 2019 / Published online: 26 November 2019

Abstract

Raphidiopsis raciborskii is the dominant species in several subtropical aquatic ecosystems. This study addressed the influence of nutrient concentration on cyanobacteria growth and saxitoxin synthesis. Therefore, we performed bioassays of interaction between the Chlorophyceae *Monoraphidium contortum* and *R. raciborskii* simulating oligotrophic and supereutrophic environments. Experiments were carried out in a climatized room for 15 days in pure cultures of each species (control) and mixed (interaction). The biomass growth (biovolume) and specific growth rates were measured. Saxitoxin was analyzed using the ELISA biochemical method. In the oligotrophic environment, the cell volume of *R. raciborskii* decreased. This species also showed senescence in interaction with *M. contortum*. However, there were no statistical differences in the saxitoxin synthesis in both conditions. In the supereutrophic environment, the growth of *R. raciborskii* and saxitoxin production was similar in both the control and interaction conditions. However, *M. contortum* growth decreased in interaction with the cyanobacteria. The increase in trophic status from oligo- to supereutrophic contributes to the growth of *M. contortum* in subtropical aquatic ecosystems, but the decrease in the area/volume ratio of cyanobacteria and the saxitoxin synthesis is an evident survival strategy in oligotrophic environments.

Keywords *Cyanobacteria* · Chlorophyceae · Trophic statuses · Competition · Cyanotoxin · Cell volume

Introduction

Cyanobacteria are the predominant organisms in tropical and subtropical aquatic ecosystems (Burford et al. 2007; Bittencourt-Oliveira et al. 2014; Casali et al. 2017) regardless of their trophic status, and *Raphidiopsis raciborskii* (Aguilera et al. 2018) are dominant in these ecosystems. Changing the nitrogen/phosphorus ratio limits the development and growth of some phytoplanktonic species, including cyanobacteria (Wetzel 1993; Kim et al. 2007). However, the amount of nitrogen and phosphorus is more important at predicting biomass accumulations. *Raphidiopsis raciborskii* can adapt to a range of temperature and light conditions and can dominate many environmental scenarios, which could explain the predominance of this species in different physical and chemical conditions (Piccini et al. 2011; Bonilla et al. 2012). Moreover, this species has competitive advantages due to its ability to migrate in the water column, low luminosity tolerance, resistance to herbivory, nitrogen fixation, high photosynthetic activity, use of dissolved inorganic carbon, and phosphorus storage (Padisák 1997; Chellappa et al. 2008; Wu et al. 2009).

The dominance of *R. raciborskii* species in freshwater ecosystems causes concern due to the synthesis of cylindrospermopsin (hepatotoxin) and saxitoxin (neurotoxin) that affects the multiple uses of water resources (Carmichael 1994; Harke et al. 2016). In general, the reasons for toxin synthesis have not been completely explained, but they may have protective functions against predation or may be related to competition and defense and survivor strategies (Burford et al. 2014; Glober et al. 2016; Harke et al. 2016; Casali et al. 2017). However, since different species of cyanobacteria can produce multiple analogues of toxins (e.g., cylindrospermopsin,

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saxitoxin, and anatoxin), the understanding about the synthesis remains uncertain (Holland and Kinnear 2013). The synthesis of different toxins by *R. raciborskii* strains may be related to its geographic distribution (Antunes et al. 2015; Burford et al. 2016). Also, there are some evidences of the increasing of the toxin synthesis which is related to nutrient limiting (e.g., nitrogen or phosphorus) (Holland and Kinnear 2013) suggesting that the evolutive advantage of the toxin synthesis is due to the competitive advantage or physiological aide (Holland and Kinnear 2013). *Raphidiopsis raciborskii* has shown notable physiological and morphological plasticity in phosphorus-limited environments that support its competitive advantages (Amaral et al. 2014). Variables such as salinity and carbonate concentration are predictors of the saxitoxin synthesis, while the cylindrospermopsin synthesis may occur regardless of the environmental condition, and its physiological or ecological role is unclear which is related (Buford et al. 2016).

There is growing interest in studies on the interaction between cyanobacteria and other potentially competing phytoplankton species (Kearns and Hunter 2000; Rodrigo et al. 2009; Zhu et al. 2010). The species interaction exhibits a pattern of different growth strategies and metabolism (photosynthesis and respiration), as well as motility and morphology (size, shape) that support the development of some groups in certain environments (Rodrigo et al. 2009). *Monoraphidium* sp. outcompetes other species in different light and nutrient conditions exhibiting metabolic traits that address responses to resource acquisition (Bestion et al. 2018). *Monoraphidium contortum* (Thuret) Komárková-Legnerová is a chlorophyte belonging to codon X1 (Padisák et al. 2009), characterized by shallow water algae and the transition from eutrophic to hypereutrophic conditions. This species belongs to the C-strategist group, with a high area/volume ratio and high metabolic activity, and under ideal conditions, they tend to exclude other species (Reynolds 1988).

The aim of this study was to evaluate the influence of trophic statuses on *R. raciborskii* growth and its saxitoxin synthesis in interaction with *M. contortum*. This *Chlorophyceae* species is dominant in many subtropical reservoirs, and in our study area, it coexists with *R. raciborskii* (Cunha et al. 2012; Beghelli et al. 2016; Casali et al. 2017). The coexistence of *R. raciborskii* and *M. contortum* in a subtropical reservoir showed a decrease of saxitoxin concentration, but it increased in periods with high concentration of inorganic dissolved nutrients when the density of *R. raciborskii* was also high (Casali et al. 2017). Considering the artificial eutrophication is occurring throughout the world and the phosphorus loading is the main cause of this process (Dodds et al. 2009) and also the main driver of cyanobacteria dominance and harmful bloom in the freshwater ecosystems (O'Neil et al. 2012), we hypothesize that high trophic status (i.e., eutrophic) supports *R. raciborskii* growth and saxitoxin production is high in low trophic status (i.e., oligotrophic) since it can be considered a defense strategy in a stressful situation. We also expect high *M. contortum* growth in oligotrophic environments. Our findings will contribute to understanding the influence of nutrient conditions on the growth of two microorganisms (Cyanobacteria and Chlorophyceae) that coexist in subtropical freshwater ecosystems and to understanding the competitive relationship that is probably an important driver of saxitoxin synthesis.

Materials and Methods

Study Area

Itupararanga Reservoir (23°36'42"S; 47°23'48"W), located in the Sorocaba River basin in São Paulo State, Brazil, is 26 km in length and has an area of approximately 936 km^2. The annual rainfall in the watershed is around 1400 mm, with dry winters and wet summers. The minimum and maximum air temperatures in the area are between 15.0 °C and 29.0 °C, respectively, and the mean wind speeds range from 1.5 to 3.0 m s^{-1} (INMET 2009, 2010). The reservoir shows spatiotemporal heterogeneity and is classified as mesotrophic and there is a recent concern about the *Raphidiopsis raciborskii* proliferation (Cunha et al. 2012; Beghelli et al. 2016; Casali et al. 2017). The main uses of the reservoir are as public water supply and power generation for an important industry. There is a permanent preservation area around the reservoir. However, agriculture (mainly strawberries, onions, potatoes, lettuce, and tomato crops) is the predominant land use in the watershed, occupying 42% of the reservoir area.

Isolation of Species and Growth Conditions

Water samples from the Itupararanga Reservoir were collected manually on the subsurface (~ 15 cm) of eight sampling stations (headwater, central axis, arms, and dam) to obtain and isolate the phytoplankton species. The samples were mixed in the laboratory in order to minimize the influence of the spatial variation.

The mixed water sample was maintained in ASM-1 culture medium (Gorham et al. 1964) to isolate *Monoraphidium contortum* and *R. raciborskii* (Fig. 1) by successive subcultures of colonies on solid medium (ASM-1 with 10% agar) using the exhaustion method until obtaining a pure colony and cycloheximide (70 mg L^{-1}) for cyanobacteria isolation (Hoshaw and Rosowski 1973; Andersen and Kawachi 2005). Two strains producing saxitoxin were isolated and three strains not producing saxitoxin were also isolated.

In this study, a single strain isolated from each species, *R. raciborskii* (ITUC01) producing saxitoxin and *M. contortum* (ITUMc01) were used. They were kept in the algae and cyanobacteria bank at the Laboratório de

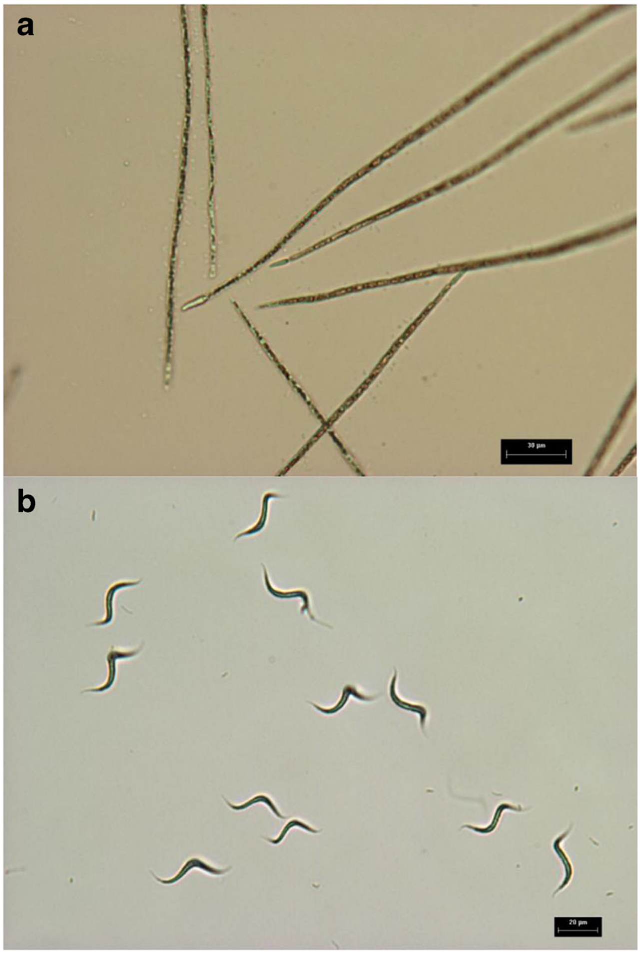

Fig. 1 *Raphidiopsis raciborskii* (A; 30 µm) and *Monoraphidium contortum* (B; 20 µm) (200 x) isolated from Itupararanga Reservoir

Biotoxicologia de Águas Continentais e Efluentes (São Carlos School of Engineering at the University of São Paulo). Both strains were maintained in an ASM-1 culture medium (pH 7.8, photoperiod, 12 h, light intensity, 60 µmol photons m^{-2} s^{-1} with a cool white fluorescent tubular lamp). During the exponential growth phase, both *R. raciborskii* and *M. contortum* were collected and cultured in incubators (25 ± 1 °C) with different nutritional conditions.

Nutritional Conditions

Water samples were collected from the Itupararanga Reservoir to determine the dissolved nitrogen (nitrite, nitrate, and ammonia) and inorganic phosphorus concentration (APHA 2005). Micronutrient concentrations (zinc, iron, manganese, copper, boron, and cobalt) were determined through atomic absorption spectrometry (AA240FS Varian) (Method 3111 B; APHA 2005). The chlorophyll-*a* concentration was determined using ethanol extraction (80% v/v) (Nuch 1980).

The results of the analysis of the water sampled from the reservoir were used to modify the ASM-1 culture medium to simulate the oligotrophic and supereutrophic conditions. The concentration of the inorganic phosphate in the water from the reservoir was 0.012 ± 0.0035 mg L^{-1}, chlorophyll-*a* concentration was 31.5 ± 1.9 µg L^{-1} and nitrate concentration was 0.45 ± 0.03 mg L^{-1}. The range of inorganic phosphate concentration in the culture medium was established according to the mean chlorophyll-*a* concentration in the reservoir, and the nitrate and micronutrient concentrations remained unchanged (Table 1).

Experimental Design

Interaction bioassays between *R. raciborskii* and *M. contortum* were performed in different nutrient concentrations to simulate the oligotrophic and supereutrophic conditions (Lamparelli 2004 modified from Carlson 1977 for tropical environments. For details see Cunha et al. 2013). For the trophic status, phosphorus concentration was assumed as inorganic phosphate concentration (Wetzel 1993).

The experiments were performed at the laboratory (total culture volume per experiment = 100 mL) for 15 days in a room under controlled temperature, photoperiod and light intensity (24 ± 0.5 °C; 12 h; 60 µmol photons m^{-2} s^{-1}, respectively), and pH 7.8. The control condition consisted of cultivating each species growing isolated in oligotrophic and supereutrophic conditions ($n = 12$). The interaction bioassays between *R. raciborskii* and *M. contortum* were carried out in both oligo- and supereutrophic conditions ($n = 6$).

To acclimatize the algae cultures, three successive subcultures were carried out during the exponential growth of each species growing in different nutrient conditions.

To obtain the similar biomass of the algae and cyanobacteria species at the beginning of the experiments, the amount of inoculum of each species used in the different experimental conditions was obtained from the calculation of biovolume (Hillebrand et al. 1999) derived from acclimatized cultures, in the exponential phase of growth, in each nutrient condition (the inoculum did not exceed 10% of the volume of the culture) (Table 2).

Every 2 days, 2.5 mL was sampled from each sample (under aseptic conditions), and aliquots (500 µL) were stored in amber glasses and fixed with acetic Lugol solution (1%) for cell density analyses (cells mL^{-1}) using Fuchs-Rosenthal chamber (APHA 2005), for cell volume analyses (μm^3) (30 organisms per species) (Hillebrand et al. 1999) and for the biovolume calculation (μm^3 mL^{-1}). Aliquots (2 mL) were kept in glasses and were frozen (-20 °C) for saxitoxin analysis.

From the exponential growth of both species, the specific growth rate (µ) was calculated (Stein 2003). The calculation of carbon biomass was performed based on the cell volume of the species (Rocha and Duncan 1985) and biovolume of each algae species in each sampling day (µg C L^{-1}).

Table 1 Nutrient and micronutrient concentrations used to simulate the oligotrophic and supereutrophic conditions and trophic state index (TSI) (Lamparelli 2004)

Trophic state	Inorganic phosphate (mg L^{-1})	Nitrate (mg L^{-1})	Micronutrients (mg L^{-1})		TSI
Oligotrophic	0.0033	0.45	Zinc Iron Manganese	0.0875 0.2425 0.021	49.5
Supereutrophic	0.0353		Copper Boron Cobalt	0.0135 0.9 0.0075	63.9

The toxin was extracted through four freeze-thaw cycles to cell lyse (Törökne et al. 2004; Yilmaz et al. 2008; Berry and Lind 2010) and measured using the ELISA biochemical method (μg L^{-1}).

Statistical Analysis

To compare the species growth, saxitoxin synthesis, and cell volume in each nutrient condition, we performed repeated measures ANOVA and Bonferroni post hoc test. The same test was utilized to compare the saxitoxin production between the oligotrophic and supereutrophic conditions. To compare the growth differences between the oligotrophic and supereutrophic conditions, the analysis of variance (ANOVA) test and post hoc Tukey test were used. The analyses were carried out using Origin Pro 8.0 with a confidence level of 95% ($p \leq 0.05$).

The relationship between biovolume and carbon biomass was obtained using Pearson correlation (Microsoft Excel): low correlation ($0 \leq r \geq 0.3$), moderate correlation ($0.3 < r \geq 0.6$), high correlation ($0.6 < r \geq 0.9$), and super high correlation ($r > 0.9$) (Callegari-Jacques 2003).

Results

Growth and Carbon Biomass

The mean initial biovolume was similar for *R. raciborskii* and *M. contortum* comparing the control and interaction cultures, in both oligotrophic and supereutrophic conditions ($p > 0.05$) (Tables 3 and 4). In the oligotrophic condition the correlation between carbon biomass and the biovolume growth was super high for *M. contortum* ($r = 0.99$ in the control; $r = 0.98$ in the interaction experiments) and was from moderate ($r = 0.56$) to high ($r = 0.84$) for the *R. raciborskii* control and bioassay interactions, respectively, according to the correlation proposed by Callegari-Jacques (2003).

In the supereutrophic environment, the correlation between carbon biomass and biovolume growth was super high in the *M. contortum* cultures ($r = 0.98$ in the control condition; $r = 0.99$ in the interaction bioassay). For *C. raciborskii*, the correlation was moderate ($r = 0.53$ and $r = 0.45$ in the control and in the interaction experiment, respectively).

In the oligotrophic environment, the biovolume of *M. contortum* was significantly higher in the control experiments than in the interaction with *R. raciborskii* (Fig. 2) ($p \leq 0.02$ from day 4 to 15) with interaction effect ($F_{(1,4)} = 280.8$). Moreover, the *M. contortum* specific growth rate was statistically different between the control and the interaction experiments ($p = 0.05$) (Table 5).

The *R. raciborskii* growth decreased in the interaction bioassays ($F_{(1,4)} = 69.57$) from day 6 to day 15 ($p \leq 0.01$). Furthermore, the cell volume significantly decreased ($F_{(1,58)} = 80.02$; $p \leq 0.0001$) from day 6 in the interaction assays, while in the control condition, the reduction occurred at the end of the experiment. There was no statistical difference of the specific growth rate in the control condition (Table 5). The chlorophyte showed competitive advantages over cyanobacteria. The *M. contortum* biovolume was significantly higher than the *R. raciborskii* biovolume from day 7 ($F_{(1,4)} = 297.6$; $p \leq 0.018$), as well as the specific growth rate (Table 5).

Table 2 Initial biovolume averages of *M. contortum* (Mc) and *R. raciborskii* (Rr) (10^5 μm^3 mL^{-1}), with standard deviations (***n*** = 3)

Biomass: biovolume (10^5 μm^3 mL^{-1})				
Trophic state	Cultures			
	Mc control	Mc interaction	Rr control	Rr interaction
Oligotrophic	2.00 ± 0.22	2.39 ± 0.29	2.52 ± 0.27	2.72 ± 0.16
Supereutrophic	10.59 ± 0.86	10.47 ± 0.88	11.03 ± 1.61	10.35 ± 0.91

Cultures: *Monoraphidium contortum* Control, Mc control; *Monoraphidium contortum* interaction, Mc interaction; *Raphidiopsis raciborskii* control, Rr control; *Raphidiopsis raciborskii* interaction, Rr interaction

Table 3 Biovolume ± standard deviation (SD) and carbon biomass ± SD in the *M. contortum* control and interaction bioassays at the beginning and at the end of the experiments

Nutrient condition	Period	Cultures			
		Control		Interactions	
		Biovolume (10^5 µm^3 mL^{-1})	C Biomass (10^2 µgC mL^{-1})	Biovolume (10^5 µm^3 mL^{-1})	C Biomass (10^2 µgC mL^{-1})
Oligotrophic	Initial	2.00 ± 0.22	9.88 ± 1.08	2.39 ± 0.29	11.82 ± 1.46
	Final	14.90 ± 0.46	81.49 ± 2.50	9.53 ± 0.12	48.8 ± 0.62
Supereutrophic	Initial	10.59 ± 0.86	36.65 ± 2.97	10.47 ± 0.88	36.23 ± 3.06
	Final	309.42 ± 61.26	1876.68 ± 371.58	129.34 ± 9.44	598.89 ± 43.73

In the supereutrophic environment, the growth of *M. contortum* in control and interaction conditions (Fig. 3) was significantly different ($F_{(1,4)} = 72.21$) from day 6 ($p \leq 0.01$). However, there were no statistical differences on the specific growth rate (Table 5). For *R. raciborskii* the statistical analysis showed no difference between control and interaction ($F_{(1,4)} = 6.018$; $p > 0.05$). Specific growth rates and cell volume ($F_{(1,58)} = 1.919$; $p > 0.05$) were similar in the control and interaction cultures (Table 5). In the interaction experiments the *M. contortum* biovolume was significantly higher than the *R. raciborskii* biovolume mainly from day 3 ($F_{(1,4)} = 899.3$; $p \leq 0.0001$) and the specific growth rate (Table 5).

There was higher growth rate and higher biomass yield of *M. contortum* in supereutrophic conditions than in the oligotrophic condition (Table 3) in both control and interaction. However, *R. raciborskii* showed the highest growth rate (Table 5) and the highest biomass in oligotrophic environment despite the senescence of the cyanobacteria at the end of the experiment in the interaction with *M. contortum* (Fig. 2).

R. raciborskii Saxitoxin Synthesis

Although the saxitoxin synthesis increased from day 0 to day 3, there were no temporal significant differences in the control and interaction in both oligotrophic and supereutrophic statuses (Table 6).

The concentration of saxitoxin was significantly higher in the oligotrophic condition than in supereutrophic in both control ($F_{(1,4)} = 16.57$; $p = 0.015$) (day 3; $p \leq 0.013$) and interaction experiments ($F_{(1,4)} = 16.23$; $p = 0.016$) (day 3; $p \leq 0.035$).

Discussion

Microorganisms have physiological and morphological strategies to adapt in a range of environmental conditions. Species with a high area/volume ratio improve the nutrient absorption, but organisms with low ratios can store nutrients, such as Chlorophyceae and cyanobacteria, respectively (Reynolds 2006). In the oligotrophic condition, *M. contortum* showed higher growth rate than *R. raciborskii*. In general, *M. contortum* carbon biomass in both control and interaction increased in the same magnitude order in the oligotrophic and supereutrophic conditions, but the biovolume of this species was high in the supereutrophic status. In addition, *R. raciborskii* increased its growth rate, its biovolume, and its saxitoxin synthesis in oligotrophic condition due to adaptation of this species in phosphorus-limited systems. Phosphorus-limited environments change the trichome size of *R. raciborskii* and favor physiological strategies of survival (Amaral et al. 2014).

In the supereutrophic environment, *M. contortum* growth was inhibited in the interaction experiments and had no influence on the growth of *R. raciborskii* in both the control and interaction conditions. However, the Chlorophyceae biovolume was higher than the cyanobacteria despite the highest carbon

Table 4 Biovolume ± SD and carbon biomass ± SD in the *R. raciborskii* control and interaction bioassays at the beginning and at the end of the experiments

Nutrient condition	Period	Cultures			
		Control		Interaction	
		Biovolume (10^5 µm^3 mL^{-1})	C Biomass (10^2 µgC mL^{-1})	Biovolume (10^5 µm^3 mL^{-1})	C Biomass (10^2 µgC mL^{-1})
Oligotrophic	Initial	2.52 ± 0.27	302.26 ± 33.08	2.72 ± 0.16	326.75 ± 19.55
	Final	24.60 ± 3.08	1897.13 ± 237.72	1.98 ± 0.52	144.23 ± 38.20
Supereutrophic	Initial	11.03 ± 1.61	2925.91 ± 427.05	10.35 ± 0.91	2745.43 ± 240.92
	Final	32.45 ± 3.74	5845.77 ± 674.00	24.61 ± 4.80	4213.79 ± 822.50

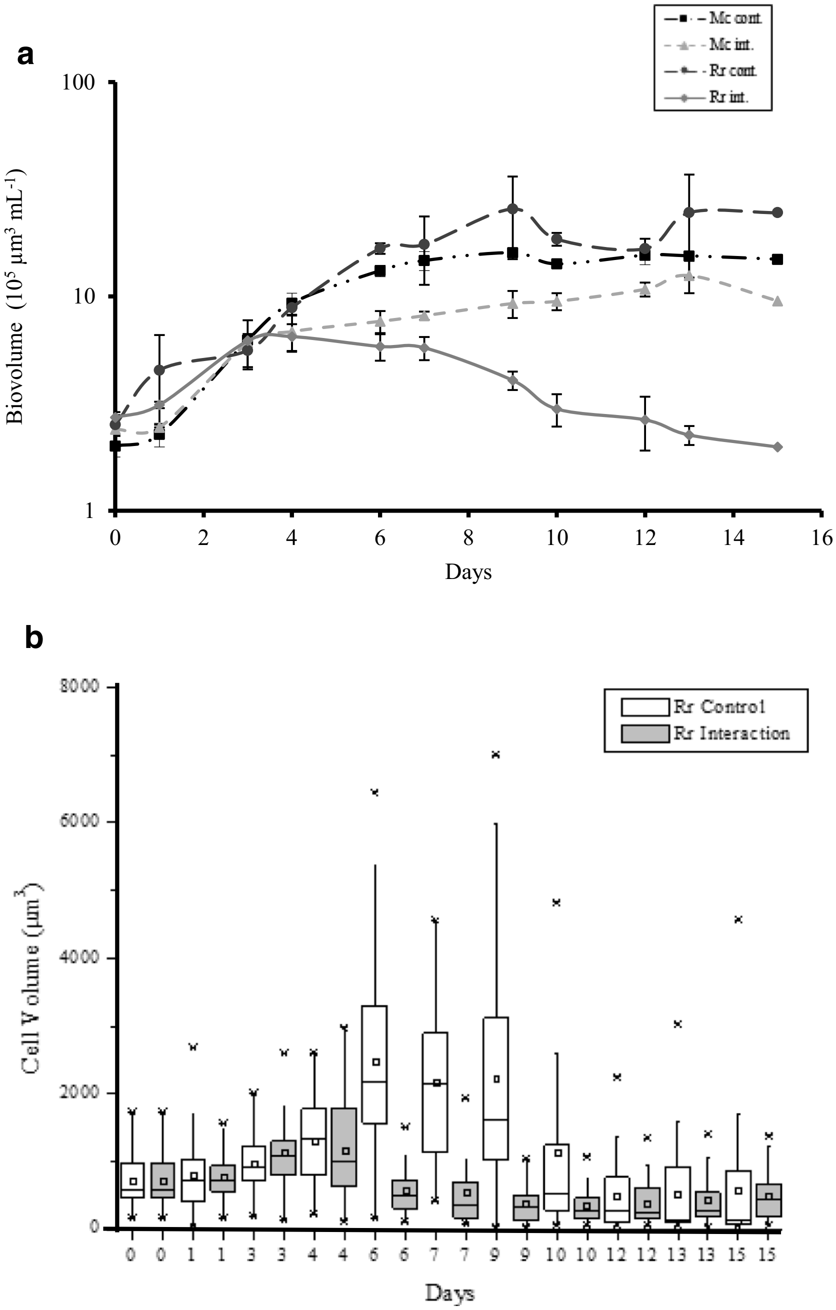

Fig. 2 Oligotrophic condition **A** Growth of *R. raciborskii* and *M. contortum* (based on mean biovolume, 10^5 µm^3 mL^{-1}). Mc cont.: *M. contortum* culture in the control experiment; Mc int.: *M. contortum* culture in the interaction experiment; Rr cont.: *R. raciborskii* culture in the control experiments; Rr int.: *R. raciborskii* culture in the interaction experiments. **B** Cell volume of *R. raciborskii* (µm^3) in the control (Rr control) and interaction (Rr interaction) cultures

biomass of *R. raciborskii* in both control and interaction bioassays.

Studies on Chlorophyceae and cyanobacteria species interactions reporting the nutrient competition are scarce (Hyenstrand et al. 2000; Kearns and Hunter 2000; Rodrigo et al. 2009; Zhu et al. 2010) and absent for the species interaction reported here. From our results, the oligotrophic environment supported *R. raciborskii* growth, and the supereutrophic condition favored *M. contortum*. Similarly, *Microcystis aeruginosa* had competitive advantages over *Scenedesmus quadricauda* in low nutrient concentrations (Zhu et al. 2010). *Monoraphidium contortum* also showed higher growth in eutrophic conditions than *Limnothrix redekei* and inhibited the *L. redekei* and *Planktothrix agardhii* growth (Rodrigo et al. 2009).

We suppose that eutrophication was not the main driver of cyanobacteria since these organisms exhibit growth strategies in stressful environments (e.g., light and nutrient limitation). A range of variables may be related to the cyanobacterial bloom in temperate and tropical environments (Kim et al. 2007; Bonilla et al. 2012) to the high photosynthetic activity, dissolved inorganic carbon absorption, phosphate utilization, and low light requirements (Padisák 1997; Wu et al. 2009). Our results are interesting for bloom management since the

Table 5 Specific growth rate (μ) (d^{-1}) ± standard deviation (SD) of *M. contortum* and *R. raciborskii* in the control and in the interaction bioassays ($n = 3$)

Nutrient condition	Specific growth rate (μ) (dia^{-1})			
	M. contortum		*R. raciborskii*	
	Control	Interaction	Control	Interaction
Oligotrophic	0.35 ± 0.04	0.45 ± 0.05* †	0.28 ± 0.11B	0.24 ± 0.03C
Supereutrophic	0.68 ± 0.07A	0.58 ± 0.07†	0.07 ± 0.01	0.06 ± 0.02

**M. contortum* control and interaction differences $p = 0.05$

† *M. contortum* and *R. raciborskii* interaction differences $p \leq 0.007$

A *M. contortum* oligotrophic and supereutrophic control differences $p = 0.0003$

B *R. raciborskii* oligotrophic and supereutrophic control differences $p = 0.02$

C *R. raciborskii* oligotrophic and supereutrophic interaction differences $p = 0.0006$

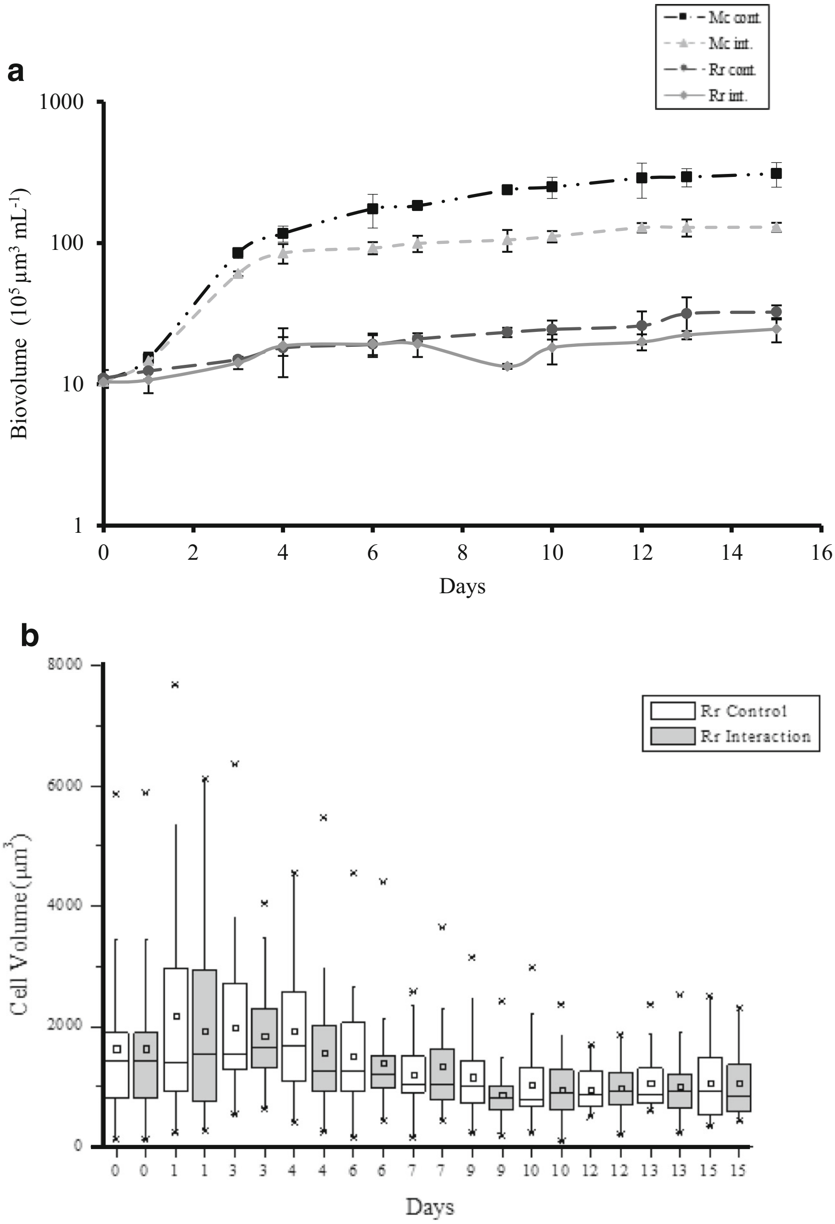

Fig. 3 Supereutrophic condition **A** Growth of *R. raciborskii* and *M. contortum* (based on mean biovolume, 10^5 µm^3 mL^{-1}). Mc cont.: *M. contortum* culture in the control experiment; Mc int.: *M. contortum* culture in the interaction experiment; Rr cont.: *R. raciborskii* culture in the control experiments; Rr int.: *R. raciborskii* culture in the interaction experiments. **B** Cell volume of *R. raciborskii* (µm^3) in the control (Rr control) and interaction (Rr interaction) cultures

Table 6 Saxitoxin concentrations (μg L^{-1}) of *R. raciborskii* control cultures (Cont.) and in interaction (Inter.), with standard deviation ($n = 3$).

Saxitoxin concentration *R. raciborskii* (μg L^{-1})						
Culture	Days					
	0	3	6	9	12	15
Oligotrophic						
Cont.	0.06 ± 0.02	0.11* ± 0.01	0.07 ± 0.006	0.09 ± 0.01	0.10 ± 0.02	0.11 ± 0.01
Inter.	0.04 ± 0.03	0.09** ± 0.04	0.08 ± 0.02	0.06 ± 0.03	0.06 ± 0.01	0.09 ± 0.01
Supereutrophic						
Cont.	0.03 ± 0.006	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.05
Inter.	0.03 ± 0.006	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.07 ± 0.02	0.06 ± 0.02

*Difference of *R. raciborskii* control between oligotrophic and supereutrophic $p \leq 0.013$

**Difference of *R. raciborskii* interaction between oligotrophic and supereutrophic $p \leq 0.035$

nitrogen/phosphorus ratio influences cyanobacteria growth (Bouvy et al. 2000; Briand et al. 2002).

Freshwater ecosystems are considered phosphorus limited for inorganic nitrogen/orthophosphate ratios higher than 7.5:1 (Lee and Jones-Lee 1998). In this research, the bioassays were carried out in phosphorus-limiting conditions. Some studies (Smith 1983; Stal et al. 2003; Kim et al. 2007) showed that the N:P ratio decrease supported the cyanobacteria growth. Also, cyanobacteria were found as dominant species in phosphorus-limited sampling site within a hypereutrophic reservoir (Kim et al. 2007). Low N:P ratio (supereutrophic condition in this study) did not affect *R. raciborskii* growth even in interactions with *M. contortum*. Therefore, *R. raciborskii* is adapted to low nutritional condition and is able to live in low N:P ratio condition and in interaction with other species. However, *M. contortum* growth was faster in supereutrophic condition (i.e., higher specific growth rate than *R. raciborskii*) indicating its advantage in nutrient-rich environments.

The saxitoxin synthesis is not only attributed to the phosphorus limitation but also to the temperature and nitrogen availability (Cirés and Ballot 2016; Glober et al. 2016). In subtropical reservoirs, the main drivers of total saxitoxin production are nutrient availability and high density of *R. raciborskii*. However, the highest saxitoxin per trichome was associated to the low species density, high temperature, total nitrogen and total phosphorus (less bioavailable), and high density of *M. contortum* (Casali et al. 2017). Therefore, the toxin synthesis is related to stressful conditions, and our outcomes pointed high saxitoxin synthesis in phosphorus-limited condition (i.e., oligotrophic). There are some evidences of the increasing of the toxin synthesis which is related to nutrient limiting (e.g., nitrogen or phosphorus) (Holland and Kinnear 2013).

The interaction between *Anabaena flos-aquae* and *Chlamydomonas reinhardtii* showed that *C. reinhardtii* growth was five times lower than in interaction experiments and 20 times less when cultivated in *A. flos-aquae* substrate. Moreover, the toxin synthesis (anatoxin and microcystin) was twice as high as the interaction experiments with Chlorophyceae (Kearns and Hunter 2000). We suppose that the saxitoxin synthesis influenced the *M. contortum* growth since it was lower in interaction condition than in the control condition in both the oligotrophic and supereutrophic environments. The high saxitoxin at the beginning of the oligotrophic experiments (until day 3) is probably due to the exponential growth phase of *R. raciborskii*.

Comparing the saxitoxin synthesis with growth rate of *R. raciborskii* (Orr et al. 2018), both oligotrophic and supereutrophic conditions showed that saxitoxin cell quota decreases probably due to saxitoxin production rate which is lower than the cell division rate or metabolic degradation ($\mu_{tox}/\mu_g < 1$: *R. raciborskii* control in the oligotrophic condition = 0.02; *R. raciborskii* interaction in the oligotrophic condition = 0.08; *R. raciborskii* control in the supereutrophic condition = 0.06; *R. raciborskii* interaction in the supereutrophic condition = 0.01). However, the saxitoxin synthesis per growth rate was high in the oligotrophic condition.

In general, in this research, the saxitoxin synthesis increasing in oligotrophic environments may be related to (i) defense and survival strategies of *R. raciborskii* and (ii) changing in the growth pattern of *R. raciborskii* in interaction experiments due to the cell volume and biomass decrease, anticipation of the stationary phase, and induction of senescence from 8 days of bioassays, as mentioned in Kearns and Hunter (2000), Rodrigo et al. (2009), and Casali et al. (2017). These factors are in agreement with Holland and Kinnear (2013) who report that the toxin synthesis is related to the physiological and competitive advantages.

Our outcomes showed that high nutrient availability supported the competitive advantages of *M. contortum*, although its growth was affected in the interaction condition. The chlorophyte did not inhibit the *R. raciborskii* growth even in supereutrophic condition, and the species coexist in nutrient-rich conditions. However, the oligotrophic condition supported *R. raciborskii* growth.

However, environmental conditions (e.g., light, pH, macro and micro nutrient concentrations, seasonality) and interaction with other species (competition, predation) and both acting together influence the dominant phytoplankton community in a reservoir (Wetzel 1993; Zhou et al. 2016). In tropical and subtropical reservoirs, these conditions change both seasonally and daily allowing the coexistence of *R. raciborskii* and *M. contortum*. Therefore, the nutrient availability is not the only driver of cyanobacteria dominance, but in a transition scenario from mesotrophic to eutrophic condition with constant cyanobacteria blooms as in Itupararanga Reservoir, phosphorus concentration is a good predictor for management.

Conclusion

Supereutrophic condition supported *M. contortum* growth and showed no effect in the *R. raciborskii* growth. However, the interaction experiments showed that the high nutrient availability reduced Chlorophyceae biomass in the presence of cyanobacteria. The *R. raciborskii* growth was higher in oligotrophic environment than in supereutrophic status, but it decreased in the interaction with *M. contortum*. Therefore, *M. contortum* and *R. raciborskii* coexist in supereutrophic subtropical environments, but the Chlorophyceae has competitive advantages over the cyanobacteria in both nutrient conditions.

Saxitoxin synthesis was constant but higher under oligotrophic condition than under supereutrophic condition, indicating a survivor strategy of the cyanobacteria and adaptation in the phosphorus-limited environment. Therefore, we reject our hypothesis that supereutrophic status supports *R. raciborskii* growth, while oligotrophic condition promotes high *M. contortum* growth. However, the low nutrient availability may be considered a response for stressful condition. The outcomes raise the understanding about trophic statuses and toxin synthesis in subtropical ecosystems and contribute to water resources management.

Acknowledgments We would like to thank the thematic project team from *Escola de Engenharia de São Carlos da Universidade de São Paulo* (EESC-USP), *Universidade Federal de São Carlos*-Campus Sorocaba (UFSCar Sorocaba), *Universidade Federal do ABC* (UFABC), and *Universidade Paulista* Campus Sorocaba (Unesp Sorocaba).

Funding information We would like to thank the *Conselho de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP) (process 2008/55636-9) for the financial support.

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