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Supplementary Material: Influence of Environmental Factors on Occurrence of Cyanobacteria and Abundance of Saxitoxin-Producing Cyanobacteria in a Subtropical Drinking Water Reservoir in Brazil

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Table S1. Characteristics of the Itupararanga reservoir and sampling sites (riverine and dam zones).

Characteristics	Itupararanga	
Surface area (km²)	20.9	
Maximum volume (10^6 m³)	286.0	
Perimeter (km)	192.8	
Maximum water flow (m³ s^{-1})	39.0	
Water residence time (days)	250	
Main tributaries	Sorocamirim and Sorocabuçu rivers	
Hydrographic basin	Sorocaba and Middle Tietê River	
Characteristics	**Riverine zone**	**Dam zone**
*Geographic location	23°37′24.8″ S, 47°13′53.4″ W	23°36′50.7″ S, 47°23′27.3″ W
*Maximum depth (m)	6.5	18.5
*Average depth (m)	5.0	16.5

Source: Cunha and Calijuri [29]; Cunha [103]; Beghelli et al. [30]; *this study.

Table S2. List of phytoplankton pigments included in HPLC analysis and taxonomic designations.

	Pigment	Designation
1	α-carotene	
2	Alloxanthin	Cryptophytes
3	Antheraxanthin	Green algae
4	Aphanizophyll	Cyanobacteria
5	β-carotene	
6	Canthaxanthin	Cyanobacteria
7	Chlorophyll a	
8	Chlorophyll b	Green algae
9	Chlorophyll c2	
10	Chlorophyll c3	Diatoms and chrysophytes
11	Chlorophyllide a	
12	Diadinoxanthin	
13	Diatoxanthin	
14	Echinenone	Cyanobacteria
15	Fucoxanthin	Diatoms and chrysophytes
16	Lutein	Green algae
17	Myxoxanthophyll	Cyanobacteria
18	Neoxanthin	Green algae
19	Peridinin	Dinoflagellates
20	Pheophytin a	
21	Violaxanthin	Green algae
22	Zeaxanthin	Cyanobacteria

Table 3. PCR and *in silico* PCR with cyanobacteria strains for investigation of specificity of the primer set *sxtA*-cyano on detection of *sxtA* gene (+ and – indicate positive or negative PCR reaction, respectively).

Strains	PCR	In silico PCR	Strain procured from
Aphanizomenon gracile K-0549	+		SCCAP, Denmark
Dolichospermum lemmermannii K-0584	+		SCCAP, Denmark
Microcystis aeruginosa CCMA-UFSCar 666	–		CCMA-UFSCar, Brazil
Microcystis aeruginosa PCC-7820	–		PCC, France
Microcystis wesenbergii NIES-107	–		NIES, Japan
Raphidiopsis raciborskii CYRF-1	+		LETC, Brazil
Raphidiopsis raciborskii T3	+		LETC, Brazil
Raphidiopsis raciborskii CYLP-1	–		LETC, Brazil
Raphidiopsis raciborskii NPCS-1	–		LETC, Brazil
Aphanizomenon gracile		+	
Aphanizomenon ovalisporum UAM287		+	
Dolichospermum circinale		+	
Dolichospermum flos-aquae ANA311E		+	

Lyngbya wollei	+
Raphidiopsis raciborskii	+
Heteroscytonema crispum	+

SCCAP = Scandinavian Culture Collection of Algae and Protozoa; CCMA-UFSCar = Collection of Freshwater Microalgae Cultures of Federal University of São Carlos; PCC = Pasteur Culture Collection; NIES = National Institute for Environmental Studies; LETC = Laboratory of Ecophysiology and Toxicology of Cyanobacteria of Federal University of Rio de Janeiro.

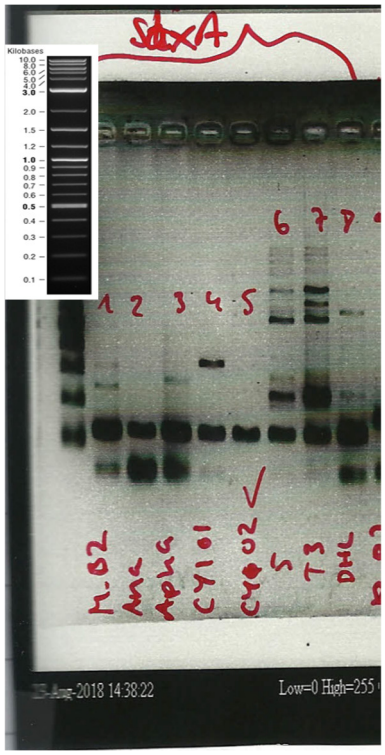

Figure S1. Agarose gel picture showing the PCR amplicons against 2-log ladder. The PCR was performed using primer pair reported by Al-Tebrineh et al. [15] for targeting the *sxtA* gene. DNA from several saxitoxin-producing cyanobacteria was used as template. As can be seen on the gel picture, a non-target amplification occurred for some strains when using the *sxtA* primer set by Al-Tebrineh et al. [15].

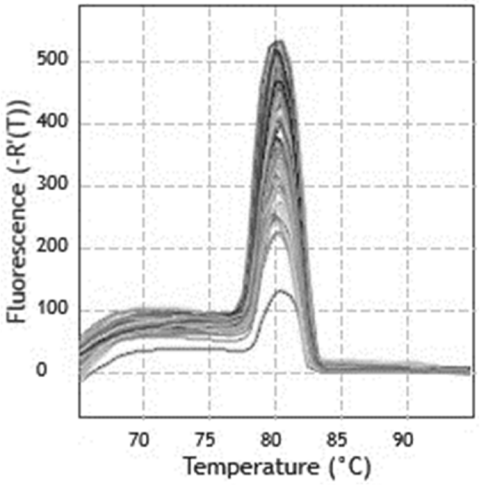

Figure S2. Melting curve for the *sxtA* primer set for standards and environmental samples showing a single peak (T_m = 80 - 80.5 °C) that indicates amplification of a single amplicon. T_m = melting temperature.

Table 4. List of the phytoplankton taxa identified in Itupararanga reservoir.

	Taxa	Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
Chrysophytes		+	+	+	+	+	+	+	+			+				+	+
1	Chromulina elegans Doflein 1921	+	+	+	+	+	+	+	+								
2	Dinobryon divergens O.E. Imhof 1887							+	+							+	+
3	Mallomonas sp.							+									
4	Synura sp.			+					+			+					
Cryptophytes		+		+	+	+	+	+	+	+	+	+	+		+	+	+
5	Cryptomonas brasiliensis A. Castro, C.E.M. Bicudo & D. Bicudo 1992							+		+		+				+	
6	Cryptomonas erosa Ehrenberg 1832	+		+	+	+	+	+	+	+	+	+	+			+	+
7	Rhodomonas lacustris Pascher & Ruttner 1913			+	+		+	+	+						+		
Cyanobacteria		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Aphanizome				+		+			+	+	+	+		+		+

	Taxa	Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
	non gracile Lemmerman 1907																
9	Aphanocapasa incerta (Lemmerman) G.Cronberg & Komárek 1994			+	+			+	+								
10	Aphanocapsa delicatissima West & G. S. West 1912			+						+		+	+		+		
11	Aphanocapsa elachista West & G.S. West 1894				+	+	+	+	+	+	+	+	+	+	+	+	+
12	Aphanocapsa sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	Aphanothece sp.													+		+	
14	Chroococcus sp.														+		
15	Cuspidothrix sp.										+	+	+				
16	Dolichospermum solitarium (Klebahn)					+	+	+	+							+	+

	Taxa	Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
	Wacklin et al. 2009																
17	Geitlerinema sp.				+			+		+	+	+		+	+	+	+
18	Geitlerinema amphibium (C.Agardh ex Gomont) Anagnostidis 1989							+		+	+	+	+	+	+	+	+
19	Glaucospira sp.				+			+	+			+	+				
20	Limnococcus limneticus (Lemmermann) Komárková et al. 2010					+										+	+
21	Limnothrix planctonica (Woloszynska) Meffert 1988	+		+	+	+	+		+	+	+	+	+	+	+	+	+
22	Merismopedia punctata Meyen, nom. illeg. 1839															+	
23	Merismopedia tenuiss	+	+						+								

	Taxa	Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
	ima Lemmerman n 1898																
24	Microcystis protocystis W.B. Crow 1923							+									
25	Phormidium sp.										+						
26	Pseudanabaena catenata Lauterborn 1915	+	+														
27	Pseudanabaena limnetica (Lemmerman) Komárek 1974	+	+	+	+	+	+	+	+								
28	Raphidiopsis raciborskii (Wolos zynska) Aguilera et al. 2018	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	Synechocystis aquatilis Sauvageau 1892	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diatoms		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

	Taxa	Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
30	Aulacoseira granulata (Ehrenberg) Simonsen 1979	+		+	+			+	+			+					+
31	Cyclotella meneghiniana Kützing 1844	+	+	+	+	+	+	+	+	+	+					+	
32	Discostella stelligera (Cleve & Grunow) Houk & Klee 2004			+	+										+		+
33	Encyonopsis sp.	+	+				+					+					
34	Fragilaria aquaplus Lange-Bertalot & S. Ulrich 2014							+									
35	Fragilaria gruno wii Lange-Bertalot & S. Ulrich 2014	+	+	+	+	+	+			+	+	+	+	+	+	+	+
36	Fragilaria longifusiformis						+	+	+						+		+

Taxa		Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
	(Hains & Sebring) Siver et al.																
37	Fragilaria spectra P.D.Almeida, E.Morales & C.E.Wetzel 2016	+		+	+		+			+	+		+				
38	Navicula sp.	+	+														
39	Pinnularia sp.	+			+												
Dinoflagellates		+				+	+	+	+		+	+	+				
40	Ceratium furcoides (Levander) Langhans 1925	+				+	+				+		+				
41	Peridinium sp.							+	+			+	+				
Euglenophytes		+	+	+	+	+	+	+	+		+	+	+	+	+		+
42	Euglena sp.	+				+		+	+		+		+				
43	Phacus sp.							+									
44	Trachelomonas spinosa A. Stokes 1890		+														
45	Trachelomonas volvocina (Ehren	+	+	+	+	+	+	+	+		+	+	+	+	+		+

	Taxa	Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
	berg) Ehrenberg 1834																
Green algae		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
46	Actinastrum sp.	+															
47	Acutodesmus acuminatus (Lagerheim) P. M. Tsarenko 2000										+						
48	Ankistrodesmus fusiformis Corda 1838						+		+	+	+	+	+	+	+	+	+
49	Ankistrodesmus gracilis (Reinsch) Korshikov 1953							+									
50	Chlamydomonas sp. 1	+		+	+	+	+	+	+	+	+	+	+	+		+	+
51	Chlamydomonas sp. 2								+						+		
52	Chlorella sp.			+				+	+	+		+					
53	Closteriopsis longissima (Lemmermann) Lemm										+	+	+		+	+	

Taxa		Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
	erman n 1899																
54	Closter ium acutu m Brébiss on 1848	+										+		+	+	+	
55	Coelas trum microp orum Nägeli 1855								+								
56	Cosma rium sp.															+	+
57	Crucig enia fenestr ata (Schmi dle) Schmi dle 1900			+													+
58	Desmo desmu s sp.			+			+	+	+			+			+		+
59	Eremo sphaer a sp.									+							+
60	Golenk inia sp.							+				+	+				
61	Kirchn eriella contort a (Schmi dle) Bohlin 1897			+	+		+		+				+		+		
62	Kirchn eriella lunaris (Kirch ner) Möbiu s 1894										+		+				
63	Micrac tinium pusillu	+	+						+				+				

	Taxa	Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
	m Freseni us 1858																
64	Monor aphidi um arcuat um (Korsh ikov) Hindá k 1970			+	+		+	+	+				+				+
65	Monor aphidi um contort um (Thure t) Komár ková-Legner ová 1969			+		+		+	+	+	+	+	+	+	+	+	+
66	Monor aphidi um irregul are (G.M.S mith) Komár ková-Legner ová 1969									+		+	+	+	+		+
67	Monor aphidi um komar kovae Nygaa rd 1979			+			+	+		+		+	+		+	+	+
68	Monor aphidi um minut um (Nägel i) Komár		+				+			+							

Taxa		Riverine zone								Dam zone							
		May 17		Aug 17		Oct 17		Jan 18		May 17		Aug 17		Oct 17		Jan 18	
		100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
	ková-Legnerová 1969																
69	Mougeotia sp.			+		+	+	+	+		+	+	+			+	+
70	Oocystis sp. 1		+	+				+	+								
71	Oocystis sp. 2			+													
72	Pandorina morum (O. F. Müller) Bory 1826		+								+						
73	Radiococcus sp.	+	+	+	+	+	+	+	+	+	+	+	+				
74	Staurastrum chaetoceras (Schröder) G.M.Smith 1924							+								+	+
75	Staurastrum sp.										+						
76	Tetraëdron incus (Teiling) G.M. Smith 1926									+							+
77	Treubaria sp.											+					

100 = surface; 1 = lower limit of euphotic zone; + = presence of the taxon at the sampling point.

Environmental variables

The measured environmental variables varied significantly between sites ($p < 0.05$), except for dissolved oxygen, water temperature, ammonium and chlorophyll *a*, but only water temperature varied significantly between months ($p = 0.003$) (Table S5). The turbidity was highest in the riverine zone (i.e., transparency and Z_{eu} were low), reducing the light penetration in the water column.

Among the spatial variables, pH was slightly higher (pH 7.18) in the dam zone than in the riverine zone (pH 6.71), while the mean conductivity was higher in the riverine zone (83.50 μS cm^{-1}) than in the dam zone (68.13 μS cm^{-1}). Water temperatures peaked in October 2017 and January 2018 (22.5 °C and 24.2 °C, respectively) and were lowest in May and August 2017 (19.0 °C and 16.5 °C, respectively). None of the environmental variables varied significantly between depths ($p > 0.05$).

Nutrient concentrations were higher in the riverine than in the dam zone, except for ammonium. For phosphorus, concentrations of TP were 4.5-fold higher in the riverine than the dam zone (means of 51.82 vs. 11.70 μg L^{-1}, respectively), while soluble reactive phosphorus in the riverine zone ranged from 3.28 to 7.37 μg L^{-1} but was below detection limit in the dam zone. TN mainly consisted of nitrate (79% in the riverine zone and 69% in the dam zone) with mean concentrations of 0.83 mg L^{-1} (riverine zone) and 0.36 mg L^{-1} (dam zone). Similarly, maximum nitrite occurred in the riverine zone (12.59 μg L^{-1}) as compared to the dam zone (2.18 μg L^{-1}). The highest ammonium concentration was detected in May 2017 in the dam zone (144.77 μg L^{-1}), but was undetectable in October 2017 and January 2018 at the same sampling site. The mean TN:TP ratios were higher in the dam zone (149) than in the riverine zone (37), indicating a limitation by phosphorus in the dam.

Chlorophyll *a* varied between 2.30 and 79.49 μg L^{-1} with the highest concentration detected in October 2017 in the riverine zone during a dinoflagellate bloom. The trophic state index classified the riverine zone as eutrophic and the dam zone as mesotrophic (Table S5).

Table S5. Environmental variables summarised as the mean values and ranges, and statistical results (*p* values) of non-parametric Mann-Whitney test for spatial differences (riverine and dam zone) and non-parametric Kruskal-Wallis for temporal differences (months) of the environmental variables in Itupararanga reservoir.

Variables	Riverine zone (n = 8)	Dam zone (n = 8)	Spatial factor	Temporal factor
Water transparency (m)*	1.20 (0.90 - 1.60)	1.50 (1.20 - 1.90)	-	-
Z_{eu} (m)*	2.38 (2.00 - 3.00)	5.25 (4.50 - 7.00)	-	-
pH	6.71 (6.43 - 7.04)	7.18 (6.70 - 7.73)	0.010	ns
Dissolved oxygen (mg L^{-1})	5.91 (3.18 - 8.49)	7.13 (6.21 - 8.62)	ns	ns
Conductivity (μS cm^{-1})	83.50 (65.00 - 101.00)	68.13 (63.00 - 73.00)	0.014	ns
Turbidity (NTU)	10.65 (5.70 - 16.90)	5.25 (3.00 - 7.00)	0.003	ns
Water temperature (°C)	19.97 (15.47 - 25.17)	21.15 (17.10 - 24.70)	ns	0.003
Total phosphorus (TP) (μg L^{-1})	51.82 (38.70 - 70.02)	11.70 (8.90 - 15.19)	0.001	ns
Soluble reactive phosphorus (μg L^{-1})	5.64 (3.28 - 7.37)	nd	0.001	ns
Total nitrogen (TN) (mg L^{-1})	1.05 (0.83 - 1.34)	0.52 (0.38 - 0.67)	0.001	ns
Nitrate (mg L^{-1})	0.83 (0.70 - 1.04)	0.36 (0.32 - 0.42)	0.001	ns
Nitrite (μg L^{-1})	5.70 (nd - 12.59)	0.77 (nd - 2.18)	0.006	ns
Ammonium (μg L^{-1})	40.49 (15.86 - 81.67)	46.09 (nd - 144.77)	ns	ns
TN:TP	37 (24 - 60)	149 (56 - 237)	0.001	ns
Chlorophyll a (μg L^{-1})	27.65 (2.30 - 79.43)	20.03 (15.79 - 23.29)	ns	ns
Trophic state	Eutrophic	Mesotrophic	-	-

*Water transparency and Z_{eu} (lower limit of euphotic zone) (n = 4); TN:TP = total nitrogen and total phosphorus ratio; nd = not detected; ns = not significant.