

Article

Detection of Cyanotoxin-Producing Genes in a Eutrophic Reservoir (Billings Reservoir, São Paulo, Brazil)

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Received: 2 October 2019; Accepted: 13 December 2019; Published: 23 March 2020



Abstract: CyanoHABs (cyanobacterial harmful algal blooms) are blooms of cyanobacteria capable of producing cyanotoxins, a large group of secondary metabolites that are toxic to most eukaryotes. In this work, the main aim was to evaluate the presence of multiple genes from each of the clusters responsible for biosynthesis of cyanotoxins (cylindrospermopsin, microcystin and saxitoxin) in total DNA obtained from sixteen environmental water samples by PCR. Microcystin gene *mcyE* was amplified in all analyzed samples. Among the cylindrospermopsin genes analyzed, only the *cyrC* gene was amplified from DNA obtained from three of sixteen samples. Of the three different saxitoxin genes analyzed, *sxtB* and *sxtI* were present in four and three of the sixteen samples studied, respectively, and *sxtA* did not show any positive result. Based on our results, we suggest caution when using only one gene from the full clusters responsible for biosynthesis of cyanotoxins, given that it may not be sufficient to confirm or exclude the toxigenic potential of a sample.

Keywords: cyanotoxin genes; environmental monitoring; PCR

1. Introduction

Cyanobacteria are recognized as the most widespread photosynthetic organisms mainly because they show a cosmopolitan distribution and prominent ecological occupation in eutrophic environments, with the tendency to produce blooms [1,2]. Cyanobacterial blooms are considered harmful to aquatic environment because they can alter a wide range of ecosystem factors such as oxygen levels, pH and light availability. Most of the bloom-forming species are capable of producing cyanotoxins and are called cyanobacterial harmful algal blooms (CyanoHABs) [2–5].

Cyanotoxins are large groups of secondary metabolites which are toxic to most eukaryotic organisms and can be classified according to their mechanism of action: neurotoxic (anatoxin-a and analogues, saxitoxin and analogues), hepatotoxic (microcystins and nodularins), dermatotoxic (lipopolysaccharides) and cytotoxic (cylindrospermopsins) [6–8]. Some species and genera, such as *Microcystis aeruginosa*, *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique and Salerno (basonym *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju) [9], *Planktothrix* and *Dolichospermum* have been numerous reported to produce toxins [7,10–12].

Monitoring cyanobacteria and cyanotoxins is of utmost importance in water bodies used for public water supply due to the characteristics described above. Thus, several countries have regulations for

water body certification, which establish limits on the number of cyanobacterial cells and cyanotoxins levels. Cyanobacteria monitoring is carried out mainly by cell counts and is expressed in cells per mL or biovolume. The World Health Organization (WHO) suggests investigating biovolume values above $0.2 \text{ mm}^3/\text{L}$ [13]. Brazilian regulations allow cyanobacterial biovolumes of up to $10.0 \text{ mm}^3/\text{L}$ in water bodies used for public water supply following conventional treatment [14]. For cyanotoxins, WHO suggests only the limit of $1.0 \text{ }\mu\text{g}/\text{L}$ for microcystin-LR, which can be extrapolated to other microcystin variants [15]. In Brazil, limits established by legislation for cyanotoxins are $1.0 \text{ }\mu\text{g}/\text{L}$ for microcystins and $3.0 \text{ }\mu\text{g}/\text{L}$ for saxitoxins [16].

It is impossible to morphologically differentiate producing and non-producing strains [17]; hence, the presence of species known to produce cyanotoxins in blooms indicates the need for monitoring of the area to avoid harm to the environment and health of humans and animals. Due to the description of gene clusters responsible for biosynthesis of various types of cyanotoxins, it is possible to use molecular biology to evaluate the possible toxigenic capacity of cyanobacterial blooms using PCR technique. There are numerous examples of studies demonstrating a correlation between presence of genes and toxin production and the usefulness of molecular biology techniques [18–24]. Nevertheless, there is no consensus on which gene would be most appropriate to infer the presence of toxins [18,20,21,25,26]. Despite the full description of gene clusters responsible for the production of toxins, studies evaluating more than one gene are still not many when compared to studies evaluating one gene alone [11,27–35].

Billings Reservoir, the largest water resource of São Paulo Metropolitan Region (RMSP) in Brazil, has been monitored for the presence of cyanobacteria and cyanotoxins [36–41] by CETESB (Companhia Ambiental do Estado de São Paulo), an agency of the state government of São Paulo responsible for control, supervision, monitoring and licensing of pollution-generating activities. In the last available monitoring report [42], cyanobacteria were dominant in all points on the reservoir, with concomitant presence of microcystin. In addition to the monitoring conducted by CETESB using ELISA technique, the presence of cyanotoxins at Billings Reservoir was also shown by additional studies [20,37,42–44] using mass spectrometry to identify and quantify mainly microcystin; the presence of the *mcyB* gene was additionally detected in one of the studies [20]. Saxitoxin is also monitored by CETESB; among the aforementioned studies, Anjos and colleagues [20] and Moschini-Carlos and colleagues [44] have both found variants of saxitoxin; the latter also evaluated the presence of cylindrospermopsin and anatoxin, with negative results. These results point out the recurrence of cyanobacterial blooms and the presence of cyanotoxins in Billings Reservoir.

The aim of this work was to evaluate the presence of multiple genes from each of the clusters responsible for cyanotoxin biosynthesis in natural samples of Billings Reservoir, as well as cyanobacteria composition and diversity.

The results of this work showed differential amplification for the cylindrospermopsin and saxitoxin genes analyzed. Forty-one different cyanobacterial species were identified, and among these, several potentially cyanotoxin-producing species and genera were found, such as *Microcystis aeruginosa*, *Planktothrix* and *Woronichinia*. Based on our results, we suggest caution when using a single gene to infer the presence of toxins as it may not be sufficient to confirm or exclude the possibility of cyanotoxin production.

2. Materials and Methods

2.1. Area of Study

Billings Reservoir is located in the metropolitan region of São Paulo and is used for multiple purposes, from recreation to public water supply for the population of this region. Because of various blooms of cyanobacteria and deterioration of water quality, the reservoir was subdivided into five compartments (Central Body-I, Central Body-II, Taquacetuba-Bororé, Rio Grande-Rio Pequeno and Capivari-Pedra Branca) with the objective of improving management and monitoring of the area [37,44,45]. One of these compartments, named Central Body-I (CB-I), was chosen for evaluation

in this study due to its various cyanobacterial blooms with recurrent presence of potentially toxic species, such as *Microcystis aeruginosa* and genera *Plankthotrix* and *Woronichinia* [46]. In CB-1, CETESB has monitored the composition of cyanobacteria since 2005 and the presence of cyanotoxins since 2007. In the last decade, microcystin was constantly detected while saxitoxin was reported only in 2004 and 2007 [20,44,46–53].

In this work, CB-I was subdivided into four sampling points (SP1–SP4) with the objective of covering most of the area. These points were selected based on the presence of different environmental contributions (Figure 1). SP1 is near the area where two heavily polluted rivers (Pinheiros and Tietê) have their flow direction usually reversed to the reservoir in order to prevent floods in RMSP; SP2 is close to remnant vegetation area; SP3 is closely located to a dense urban occupation area; and SP4 covered the beginning of Taquacetuba Arm, where water from Billings Reservoir is transferred to Guarapiranga Reservoir, another important reservoir in RMSP, which is also used for public supply.

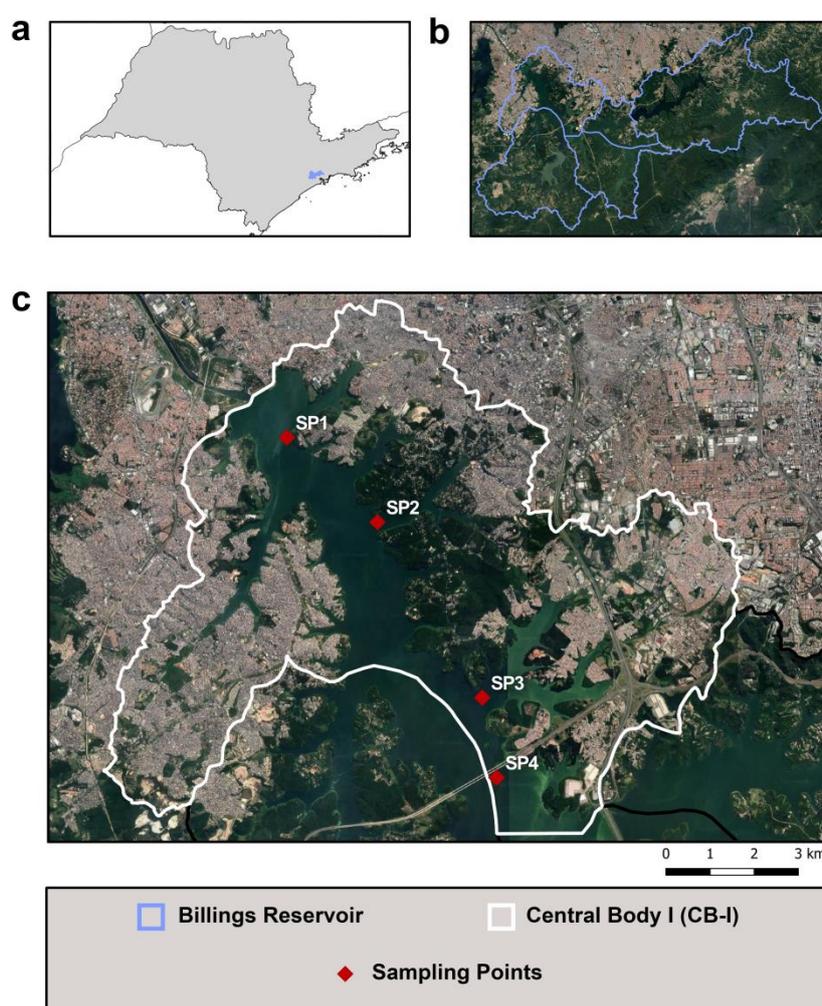


Figure 1. Location and sampling points of Billings Reservoir. (a) State of São Paulo, Billings Reservoir in blue. (b) Billings Reservoir subdivided into five compartments as per Brazilian regulation. (c) Central Body-I, sampling points in red.

2.2. Sampling

Sampling was carried out during rainy (Rainy 1—January 2017 and Rainy 2—February 2018) and dry (Dry 1—August 2016— and Dry 2—August 2017) seasons. Water samples were collected using: (a) phytoplankton net (20-micrometer mesh) for qualitative analysis for phytoplankton identification (100 mL) and DNA extraction (100 mL) and (b) Van Dorn bottle, for counts of cyanobacteria and

eukaryotic microalgae (100 mL). Both sampling techniques were performed on the surface of a water column (0.50 m). All samples were stored and transported at 4 °C until processing after no more than 2 hours from sampling.

2.3. DNA Extraction from the Microbial Community

DNA extraction was performed using PowerWater[®] DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, CA, USA), except for the initial filtration step, that was replaced by centrifugation (4.000× g for 10 min) of a sample of 30 mL. Purity, quantity and quality of each extracted DNA were evaluated by absorbance measurement at A260/280 nm and visualization at 0.8% agarose gel. DNAs samples were stored at −20 °C until the moment of PCR reactions.

2.4. Amplification of the Cyanotoxin Genes

To verify the quality of DNA extracts for amplification, these were first analyzed with primers that amplify phycocyanin operon (*cpc* gene), including a variable intergenic spacer according to the previously described protocol [54]. All primers used in this work are listed in Table 1, along with sequence, annealing temperature (AT), fragment size and reference. All PCR reactions were performed in total volume of 25 µL, which contained: 1× PCR Buffer (Invitrogen), 2 mM MgCl₂ (Invitrogen), 200 µM of each DNTP (Invitrogen), 0.4 mM of each primer, 1.5 U TAQ DNA Polymerase (Invitrogen) and 50 ng of either total extracted DNA or genomic DNA of cultured samples used as positive control. For each PCR reaction, cycling conditions available in the references of each primer were used (Table 1). All PCR reactions were performed on a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Negative control consisted of all reagents required to perform a PCR reaction without any DNA sample.

Table 1. List of primers used in this study.

| Target Gene | Primer | Sequence (5′–3′) | AT (°C) | Fragment Size (bp) | Reference |
|-------------|---------|---------------------------|---------|--------------------|-----------|
| <i>cpc</i> | PCβF | GGCTGCTIGTTTACGCGACA | 50 | 500–700 | [54] |
| | PCαR | CCAGTACCACCAGCAACTAA | | | |
| <i>cyrA</i> | CatF1 | AGATGGTGCTTATTTTGAAC | 54 | 881 | [27] |
| | CatR1 | TCITCACAGATGACCTTCTT | | | |
| <i>cyrB</i> | CPS-F | AGTATATGTTGCGGGACTCG | 55 | 478 | [55] |
| | CPS-R | CCCGCCAAGACAGAGGGTAG | | | |
| <i>cyrC</i> | CkcF3 | AATGATCGAAAAACAGCAGTCGG | 54 | 325 | [27] |
| | CkcR3 | TAGAACAATCATCCACAACCT | | | |
| <i>cyrJ</i> | cyrJ-F | TTCTCTCCTTCCCTATCTCTTATCT | 53 | 536 | [26,56] * |
| | cyrJ-R | GCTACGGTGCTGTACCAAGGGGC | | | |
| <i>mcyE</i> | mcyE-F2 | GAAATTTGTGTAGAAGGTGC | 56 | 809–812 | [57] |
| | mcyE-R4 | AATTCTAAAGCCCAAAGACG | | | |
| <i>sxtA</i> | sxtA-F | GATGACGGAGTATTTGAAGC | 55 | 125 | [18] |
| | sxtA-R | CTGCATCTTCTGGACGGTAA | | | |
| <i>sxtB</i> | SXTB-F | TTGTAGGRCAGGCACTT | 53 | 400 | [26] |
| | SXTB-R | ATCATCGGTATCATCGGTA | | | |
| <i>sxtI</i> | OCT-F | TGCCGTTTTGTGCTTAGATG | 61 | 923 | [26] |
| | OCT-R | GGACGGAAGGACTCACGATA | | | |

* Primer originally described by [56] using the amplification conditions described by [26].

2.5. Cyanobacteria Strains

Microcystis aeruginosa (NPLJ-04), *Raphidiopsis raciborskii* (T3) and *Raphidiopsis raciborskii* (CYP-011K) strains previously confirmed as microcystin, saxitoxin and cylindrospermopsin producers, respectively [26,58], were used as positive controls for PCR reactions. These strains were grown on MLA medium [59], at 12:12 h light/dark cycle and 25 ± 1 °C. DNA extraction from cultured strains was performed using the same kit and extraction method as that for microbial community. The strains

NPLJ-04, T3 and CYP-011K were kindly provided by Sandra Azevedo, Ph.D, Federal University of Rio de Janeiro.

2.6. Cyanobacteria and Eukaryotic Microalgae Identification and Density

Cyanobacteria and eukaryotic microalgae were identified by their morphometric characteristics. Phytoplankton counts were performed using sedimentation chambers (5 mL) on a Carl Zeiss Inverted Microscope, 400× augmentation, and limit criteria were as established previously by other authors [60,61]. After counting, densities were converted to biovolume (mm^3/L) using each taxon-specific biovolume from previous publications [62,63]. All samples used for counts were preserved in Lugol solution (1%), in the dark and at room temperature. Samples used for identification were preserved in formalin (4%) and stored under the same conditions described above.

3. Results

3.1. Amplification of Cyanotoxin Genes

Eight genes involved in the biosynthesis of microcystin, cylindrospermopsin and saxitoxin were evaluated by PCR from total DNA of the microbial community (Table 2 and Figure S1). The amplification reaction of phycocyanin operon was positive for all analyzed samples, confirming the presence of cyanobacterial genetic material.

Table 2. PCR results.

| Gene | DRY 1 | | | | DRY 2 | | | | RAINY 1 | | | | RAINY 2 | | | |
|-------------|-------|-----|-----|-----|-------|-----|-----|-----|---------|-----|-----|-----|---------|-----|-----|-----|
| | SP1 | SP2 | SP3 | SP4 | SP1 | SP2 | SP3 | SP4 | SP1 | SP2 | SP3 | SP4 | SP1 | SP2 | SP3 | SP4 |
| <i>cpc</i> | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| <i>mcyE</i> | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| <i>cyrA</i> | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>cyrB</i> | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>cyrC</i> | + | + | + | + | - | - | - | - | - | - | - | - | + | + | - | - |
| <i>cyrJ</i> | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>sxtA</i> | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>sxtB</i> | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>sxtI</i> | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | + |

DRY = sampling during dry periods, RAINY = sampling during rainy periods. +: positive results, -: negative results.

The microcystin gene *mcyE* was amplified in all investigated samples. Four sets of primers were used to evaluate the presence of cylindrospermopsin genes and results showed that only the *cyrC* gene was amplified from DNA of all samples collected in Dry 1 and two samples (SP1 and SP2) in Rainy 2. In this work, *sxtA*, *sxtB* and *sxtI* genes were analyzed and no amplification was observed for the *sxtA* gene. The *sxtB* gene was present in all samples in Dry 1 except for SP2, while the *sxtI* gene was amplified in only one sample in Dry 2 (SP3) and two samples in Rainy 2 (SP3 and SP4). Monitoring by CETESB provides data on microcystin concentrations collected at sampling times that correspond to those in this work. For samplings performed during dry season, microcystin concentrations were under lower limit of detection for Dry 1 ($<0.15 \mu\text{g/L}$) and $1.36 \mu\text{g/L}$ for Dry 2. For samplings carried out during rainy season, concentrations were $14.5 \mu\text{g/L}$ for Rainy 1 and $50.0 \mu\text{g/L}$ for Rainy 2 [42,46,53]. Saxitoxin was not detected in samples analyzed during this period by CETESB, and cylindrospermopsin is not part of CETESB monitoring [46,53].

3.2. Cyanobacteria Composition

Forty-one cyanobacteria species were identified, and all taxa are described in Table S1. Species *Synechocystis aquatilis*, *Aphanocapsa annulata*, *Microcystis aeruginosa*, *Planktolyngbya limnetica*, *Pseudanabaena mucicola*, *Synechococcus nidulans* and *Woronichinia naegeliana* were present in most of the samples obtained in the period under study. Species *Aphanotece zulanirae*, *Chroococcus dispersus*,

Cyanodictyon planctonicum, *Dolichospermum mucosum*, *Dolichospermum sp.*, *Dolichospermum spiroides*, *Microcystis botrys*, *Microcystis panniformis*, *Planktothrix isothrix* and *Synechococcus elongatus* were found exclusively during the dry season, while species *Aphanizomenon gracile*, *Aphanocapsa elachista*, *Aphanocapsa incerta*, *Chroococcus dispersus*, *Coelomorum pusillum*, *Eucapsis densa*, *Geitlerinema splendidum*, *Merismopedia tenuissima*, *Pseudanabaena galeata* and *Rhabdoderma sancti-pauli* were found exclusively during the rainy period. During the entire sampling period, eleven species were dominant or abundant in relation to other components of phytoplankton using biovolume as comparison unit (Figure 2), namely, *Aphanocapsa annulata*, *Coelosphaerium evidenter-marginatum*, *Dolichospermum mucosum*, *Dolichospermum nygaardii*, *Dolichospermum sp.*, *Merismopedia tenuissima*, *Microcystis aeruginosa*, *Microcystis panniformis*, *Microcystis protocystis*, *Synechocystis aquatilis*, and *Woronichinia naegeliana*.

3.3. Cyanobacteria Biovolume

Comparing sampling periods, cyanobacterial biovolume was higher in the dry season than in the rainy one (Figure 3). Furthermore, very high biovolume values were observed in this study. Such high values can be explained mainly by dominance of the species *Woronichinia naegeliana* at all points during drought and the presence of abundant species *Woronichinia naegeliana* and *Microcystis aeruginosa* during rainy periods (Figure 2).

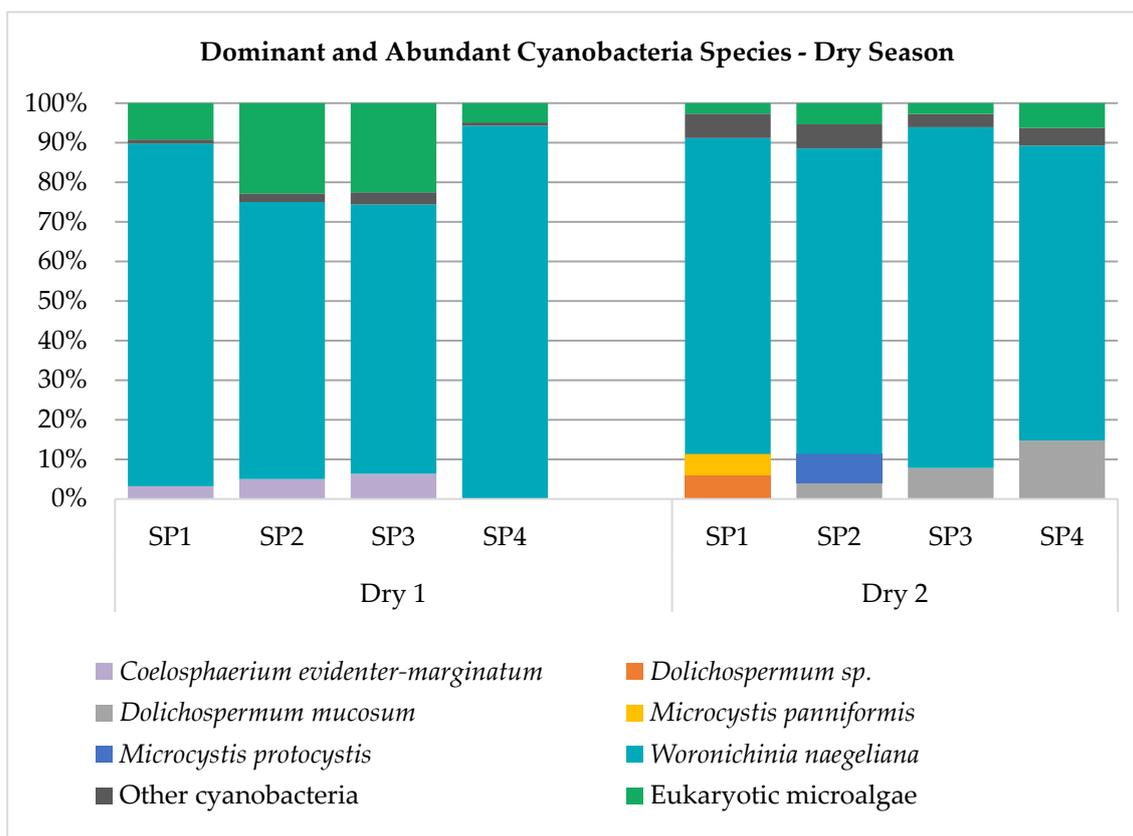


Figure 2. Cont.

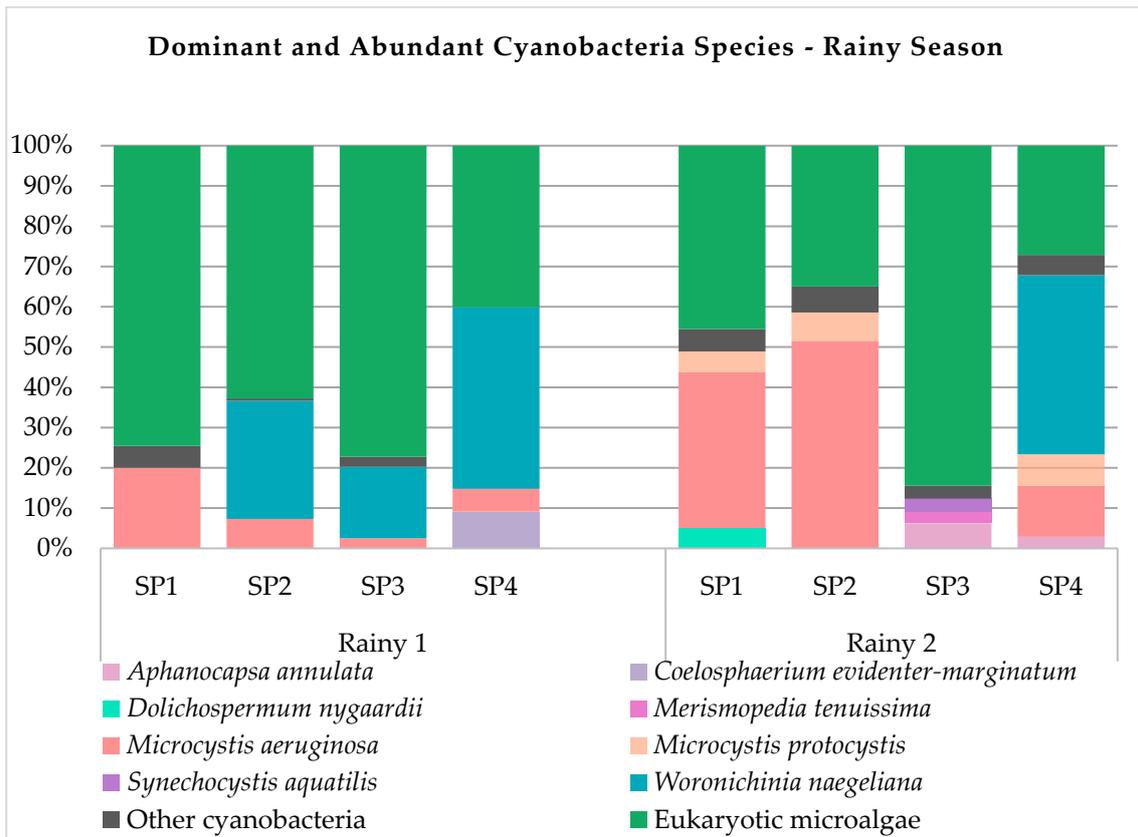


Figure 2. Dominant and abundant cyanobacteria species.

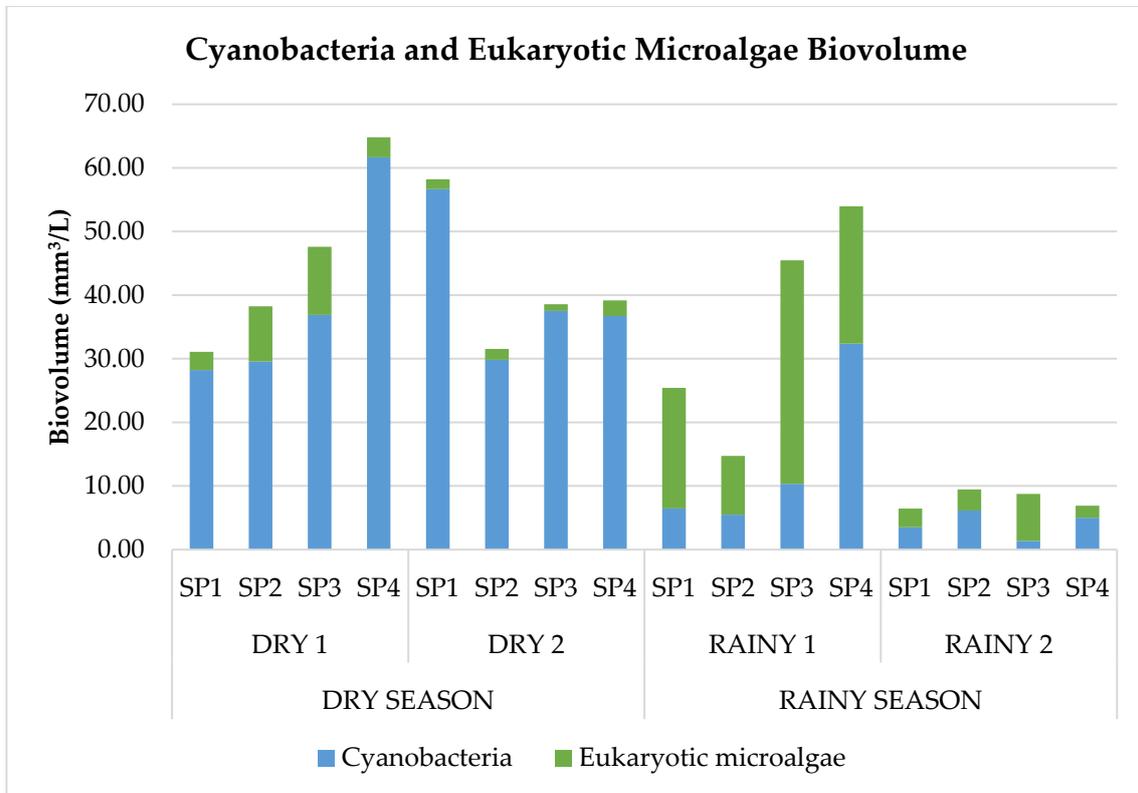


Figure 3. Cyanobacteria vs. eukaryotic microalgae biovolume.

4. Discussion

In cases of bloom events caused by cyanobacteria in reservoirs that are used for water supply, rapid discrimination between toxic (CyanoHABs) and non-toxic blooms is of great importance as basis for surveillance and management actions according to WHO recommendations [64]. Description of genetic clusters responsible for cyanotoxin biosynthesis made it possible to analyze the presence of genes and indirectly infer the presence of toxins in samples [27,57]. Hence, the use of molecular biology techniques presents itself as a very useful tool, considering that results can be obtained quickly.

There are several studies suggesting biosynthesis pathways for microcystin, saxitoxin and cylindrospermopsin, based on strains such as *Microcystis aeruginosa* PCC7806, *Aphanizomenon* sp. NH-5, *Raphidiopsis raciborskii* AWT205, *Oscillatoria* sp. PCC 6506 and *Anabaena circinalis* AWQC131C [55,56,65–67]. In this work, genes chosen for analysis were described in crucial steps of cyanotoxin biosynthesis and environmental samples studied were selected according to the monitoring data obtained by CETESB [46,52,53]. Importantly, the DNA extraction methodology applied in this study allowed the extraction of high-quality DNA that is susceptible to amplification, without inhibitors or substances that might interfere in the PCR reaction.

The detection of the *mcyE* gene in all samples could be related to the presence of microcystin in 3 of 4 samplings [42,46,53]. These data support the feasibility of using molecular biology techniques for rapid and indirect cyanotoxin monitoring in aquatic environments. The *mcyE* gene is involved in the formation and modification of the side chain of the Adda amino acid, present in all microcystin molecules, including variants. The biosynthesis pathway of this toxin has 6 more steps until completion of the molecule [67,68]. It is considered the best target as it can be amplified even in samples with low cyanobacterial biomass concentration and is also involved in crucial biosynthesis steps, supporting our findings [69,70]. Gene expression evaluation in environmental samples is the next step in further understanding whether there is a relationship between cyanotoxin concentrations in water and gene expression, considering that the detection of cyanotoxin-producing genes in total DNA samples obtained from environmental samples is still carried out through PCR technique.

Saxitoxin and cylindrospermopsin genes were detected in only some samples, but their relation to the presence of toxins in water could not be confirmed. Sets of three and four primers were analyzed for detection of genes essential for biosynthesis of saxitoxin and cylindrospermopsin, respectively, and differential amplification between genes was observed for both toxins. Genes *cyrA*, *cyrB* and *cyrC* act sequentially on cylindrospermopsin biosynthesis. The *cyrA* gene is the first to be activated and is involved in the formation of guanidinoacetate, a precursor that is activated by the next enzyme in the pathway, encoded by the *cyrB* gene. The *cyrC* gene encodes a nonribosomal peptide synthetase-polyketide synthase (NRPS-PKS) enzyme responsible for elongation of the cylindrospermopsin carbonic chain. The biosynthesis pathway of cylindrospermopsin requires tailoring reactions, i.e., reactions performed by enzymes that make additional modifications to the molecule before it is complete. The *cyrJ* gene is involved in these steps, in a sulfation reaction [65,68]. The *sxtA* gene, the first of the saxitoxin biosynthesis pathway, encodes an enzyme responsible for forming the saxitoxin precursor, 4-amino-3-oxo-guanidinoheptane, which is later converted to 4,7-diguanidino-3-oxoheptane by another enzyme (encoded by *sxtG*). The *sxtB* gene is involved in the onset of cyclization of the latter molecule, while the *sxtI* gene is involved in a transfer reaction of a carbamoyl group to the saxitoxin molecule, one of the last biosynthesis reactions in this pathway [68,71].

To date, few studies have evaluated the presence of several genes of clusters responsible for biosynthesis of microcystin, cylindrospermopsin and saxitoxin in environmental samples [11,27–35], when compared to the majority of studies in which only one gene is used. Of these, five [28,30,33–35] reported results similar to those obtained in this work, i.e., differential amplification between genes responsible for biosynthesis of a cyanotoxin. When one or more of these genes considered as crucial to the biosynthesis of cyanotoxins mutates or is deleted, synthesis of the cyanotoxin in question would be disrupted or impaired even when other genes in the cluster are present.

Accordingly, we suggest caution when using only one gene from the full cluster as it may not be sufficient to confirm the cyanotoxin production capacity in a sample. For example, a positive PCR result for the detection of a single gene within a full cluster does not necessarily entail cyanotoxin production given that other genes are needed for that (false-positive result). Moreover, if there is a silent point mutation at the primer annealing site that does not change the reading frame for transcription and translation, the mutated gene may not be detected, leading to a false-negative result. At last, there may be other biosynthetic pathways not yet known or described.

The composition of species present in Billings Reservoir was also monitored during the studied period, as a way of identifying or correlating species and genes. Some species of cyanobacteria, such as *Microcystis aeruginosa* and *Raphidiopsis raciborskii* [12,26], are constantly reported as potential cyanotoxin producers; the first one was found in samples of this study. There were no distinctive differences between composition and biovolume of cyanobacteria observed at sampling points, indicating that distribution of cyanobacteria is homogenous in CB-I of Billings Reservoir.

The species *Woronichinia naegeliiana* was dominant during most of the studied period and is reported in the literature as a potential microcystin and anatoxin-a producer [72–74]. Moreover, other species and genera that were abundant and even rare during the studied period can be related to the presence of the *mcyE* gene, such as *Microcystis aeruginosa*, *Synechocystis aquatilis*, *Dolichospermum spp* and *Aphanocapsa spp* [12,75].

In the literature, some species are constantly indicated as potential cylindrospermopsin producers, such as *Aphanizomenon aphanizomenoides*, *Aphanizomenon flos-aquae*, *Aphanizomenon ovalisporum*, *Raphidiopsis raciborskii*, *Dolichospermum lapponica*, *Umezakia natans* and *Lyngbya wollei* [7,26]. These species were not found in our work, but cylindrospermopsin genes were detected (*cyrB* and *cyrC*). This fact may suggest there are species not yet described as cylindrospermopsin producers that are responsible for biosynthesis of this cyanotoxin in Brazilian reservoirs, a hypothesis already raised before by other authors [26]. As cultivation and isolation of cyanobacteria were not performed in this study, it was not possible to verify or evaluate the relation between certain genes and species; this may serve as basis for future works.

5. Conclusions

Based on the results presented on this work, we suggest caution when using only one gene from the full cluster as it may not be sufficient to confirm the cyanotoxin production capacity. The detection of the gene in the microcystin biosynthesis cluster obtained in all samples supported the results obtained by CETESB in the same portion of Billings Reservoir indicating the presence of microcystin. Concomitant detection of cyanotoxin-producing genes and potentially cyanotoxin-producing species highlights the potential of PCR technique as a monitoring tool.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4441/12/3/903/s1>, Figure S1. PCR results; Table S1. List of species found in the study.

Author Contributions: Conceptualization, C.V.-N., C.S.F.N., M.S.F.R.; methodology, M.S.F.R., M.P.M.; validation, M.S.F.R.; formal analysis, C.V.-N., C.S.F.N., M.S.F.R., A.T.; writing—original draft preparation, C.V.-N., C.S.F.N., M.S.F.R.; writing—review and editing, C.V.-N., C.S.F.N., M.S.F.R.; supervision, C.V.-N.; project administration, C.V.-N., C.S.F.N.; funding acquisition, C.V.-N., C.S.F.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by São Paulo Research Foundation (FAPESP), grant numbers 2010/52641-1 and 2010/50738-8, and M.S.F.R. was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES), grant number (finance number) 001.

Conflicts of Interest: The authors declare no conflict of interest.

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