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Abiotic variables affect STX concentration in a meso-oligotrophic subtropical coastal lake dominated by *Cylindrospermopsis raciborskii* (Cyanophyceae)

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ABSTRACT

The cyanobacterium *Cylindrospermopsis raciborskii* is capable of producing toxins including saxitoxin (STX). Few studies have verified the influence of environmental variables on the production of STX and most have only been studied in the laboratory. The goal of this work was to identify the abiotic variables related to STX concentration *in situ*. The relationship among STX concentration and the physical variables, nutrients and chlorophyll-a (chl-a) concentration was examined in a meso-oligotrophic subtropical coastal lake dominated by *C. raciborskii*. A generalized linear model was developed, incorporating all variables measured monthly over a 45-month monitoring period. Conductivity and dissolved inorganic nitrogen (DIN) concentration provided the greatest explanatory power for STX concentration *in situ*. Previous studies suggested that *C. raciborskii* cells exposed to stress associated with higher ionic concentrations appear to activate the biosynthesis of STX suggesting that STX can elicit changes cell permeability and may contribute to the homeostasis of this organism. An increase of DIN concentration results in a higher concentration of STX which may be related to a reduced metabolic demand, since the uptake of inorganic nitrogen requires less energy than N_2-fixation. Thus, increased DIN can favor the growth of *C. raciborskii* population or improve cellular homeostasis, both potentially increasing STX concentration in the aquatic system, which was observed through a delayed response pattern. The developed model, while providing only a moderate predictive power, can assist in the understanding of the environmental variables associated with increases in STX concentration, and in monitoring and minimizing the risks of toxic blooms.

1. Introduction

The planktonic freshwater cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju 1972 (Order Nostocales) is filamentous and diazotrophic. Originally identified in tropical/subtropical regions, *C. raciborskii* has increasingly been found in temperate regions (Padisák, 1997) and is currently considered globally dispersed (Wiedner et al., 2007; Bonilla et al., 2012; Sinha et al., 2012). This cyanobacterium is considered an invasive species due to its successful global expansion, which was assisted by the ability of this species to tolerate a wide range of environmental conditions (Padisák, 1997). There is great interest worldwide in this species because it produces toxic blooms that can seriously impact water quality, thus threatening human health (Chorus and Bartram, 1999; Chorus, 2005; Burch, 2008).

The toxic secondary metabolites produced by *Cylindrospermopsis raciborskii* include the potent cylindrospermopsin (CYN) (Ohtani et al., 1992; Li et al., 2001) and saxitoxin (STX) (Lagos et al., 1999; Molica et al., 2002, 2005), which is the parent compound of more than 30 naturally occurring analogs (Kellmann et al., 2008). Furthermore, the gene locus that is responsible for the biosynthesis of STX in *C. raciborskii* has been described by Kellmann et al. (2008).

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Saxitoxins are a group of carbamate alkaloid neurotoxins that are non-sulfated (saxitoxins – STX), singly sulfated (gonyautoxins – GTX) or doubly sulfated (C-toxins). In addition, decarbamoyl variants and several new toxins have been identified in some species (Sivonen and Jones, 1999). These compounds act by blocking voltage-gated sodium channels (Kao and Levinson, 1986) and also interfere with voltage-gated calcium and potassium channels. Blockage of sodium channels results in the interruption of nervous conduction and subsequently causes muscular paralysis. Interference with voltage-gated calcium and potassium channels results in a depression of cardiac output (Carmichael, 1994; Wang et al., 2003; Su et al., 2004). Thus, STX can be highly toxic and, depending on the dose, lethal to many species, including humans (Landsberg, 2002).

The dominance of *Cylindrospermopsis raciborskii* in different freshwater systems in Brazil is related to dry periods, polymictic environments and high phosphorus concentrations (Soares et al., 2013). Positive relationships between species dominance and variables such as temperature, pH, alkalinity and conductivity have also been observed (Gomes et al., 2013). Though these studies suggest that environmental variables are related to the increase in biomass of *C. raciborskii* in freshwater systems, few studies have been performed to verify the influence of these environmental variables of the concentration of STX (Molica and Azevedo, 2009). Laboratory-based studies have examined the influence of temperature (Castro et al., 2004), N:P ratio (Chislock et al., 2014), light intensity and quality (Carneiro et al., 2009), the concentration of ions in water and pH values (Pomati et al., 2003a, 2004; Kellmann and Neilan, 2007; Carneiro et al., 2011, 2013; Ongley et al., 2016) on STX production.

Considering the current literature, no work has linked abiotic variables to STX concentration produced by *Cylindrospermopsis raciborskii* in natural systems (*in situ*). New knowledge regarding which abiotic variables influence the STX concentration is needed in order to explain an ecologically important question. As the relationship of *C. raciborskii* with higher trophic levels supports the bottom-up theory (Murdoch, 1966), the presence of this species can alter the trophic structure of freshwater systems (Leonard and Pearl, 2005). Furthermore, it is necessary to put particular focus on determining the environmental conditions required for the biosynthesis of toxins on a species-by-species basis (Merel et al., 2013). Understanding the environmental variables associated with toxin concentration to determine the conditions influencing cyanotoxins production is critical for effective lake management and minimization of health risks associated with cyanotoxins (Xie et al., 2012).

In this study, the following question was addressed: does a variation in abiotic variables determine the concentration of STX by *Cylindrospermopsis raciborskii* in the natural environment? The hypothesis is that if the biosynthesis of STX is activated in *C. raciborskii* by abiotic variables *in vitro*, this relationship will also be found *in situ*. The goal of this work was to identify which abiotic variables, if any, are related to STX concentration *in situ*.

2. Methodology

2.1. Study area

Peri Coastal Lake (27°44′ S and 48°31′ W) is located in the southeastern region of Santa Catarina Island, Brazil. The climate in the area is typically subtropical. The lake has a surface area of 5.07 km^2 and average and maximum depths of 4.2 m and 11.0 m, respectively. The volume is 21.2 million m^3 of water (Laudares-Silva, 1999; Oliveira, 2002). Peri Coastal Lake exhibits spatial homogeneity both horizontally and vertically (Hennemann and Petrucio, 2010; Tonetta et al., 2013). It is a coastal freshwater lake without marine influences (freshwater year-round), which makes it the main freshwater resource for Santa Catarina Island. Since 2000, the lake has supplied drinking water to approximately 100,000 inhabitants of Santa Catarina Island. The only other activity allowed in the lake is recreational swimming. The lake and surroundings (including almost the entire drainage basin) are within an environmentally protected area.

Peri Coastal Lake was classified as oligotrophic for nutrients concentrations and meso-eutrophic for transparency and chlorophyll-a (chl-a), which can be explained by the high densities of the cyanobacterium *Cylindrospermopsis raciborskii* and by the high recycling rates observed in tropical and subtropical water bodies (Hennemann and Petrucio, 2010). The cyanobacterium *C. raciborskii* is dominant (80% of the phytoplankton community) with high density (23 to 220×10^3 ind mL^{-1}), which results in low occurrences of other species (Tonetta et al., 2013).

2.2. Limnological variables

Peri Coastal Lake was sampled at a central point, with a maximum depth of 8.3 ± 1.3 m, at the depth of Secchi Disk extinction (0.9 ± 0.2 m). Samples were taken over a 45-month period between March 2007 and August 2014. Sub-samples were filtered (glass microfiber filter AP40 – Millipore®) for analysis of chl-a and dissolved nutrients.

The variables measured in the field were: water transparency (Secchi Disk; Chapman, 1992), water temperature, pH, conductivity and dissolved oxygen (YSI probe-85). In the laboratory, total alkalinity was determined by titration (Mackereth et al., 1978), and the concentrations of total nitrogen and total phosphorus (Valderrama, 1981) were determined from unfiltered water samples. Nitrite (Golterman et al., 1978), nitrate (Mackereth et al., 1978), ammonia (Koroleff, 1976) and soluble reactive phosphorus (Strickland and Parsons, 1960) were determined from filtered water samples. Chlorophyll-a was measured by extraction with acetone 90%, with a correction for phaeopigment (Lorenzen, 1967).

2.3. STX data

Water from Peri Coastal Lake was sampled at the water uptake point of the supply system that treats and distributes water for the population. These samples were routinely taken weekly by the Catarinense Water Supply and Sanitation Company (CASAN), which is responsible for the system.

The samples were subjected to triple freezing and thawing to analyze the concentration of STX in the whole water. High-performance liquid chromatography (HPLC) analysis of saxitoxin was carried out on the initial 80% methanolic extract using an HPLC system (Shimadzu, Japan). Saxitoxin concentration was analyzed according to the post-column derivatization method (Oshima, 1995). The chromatography was conducted at 30 °C on a Luna® 4.6 mm × 250 mm column (Phenomenex, USA) filled with 5 μm C8 particles. After the chromatography, the column eluents were derivatized with buffered 50 nM periodic acid and stabilized with acetic acid using a two-piston LC 10® pump (Shimadzu, Japan). The peaks were detected on a RF 10Alx® fluorometer (Shimadzu, Japan). The system was calibrated with a standard obtained from the National Research Council, Canada. This method was used to analyze the water samples collected through March 2013 ($n = 28$). From April 2013 onward ($n = 17$), STX concentrations were analyzed by ELISA using the Microplate Kit developed and provided by Beacon Analytical Systems Inc. Only saxitoxin was analyzed, excluding other possible analogs. The detection limit in both methods was 0.01 $\mu g\,L^{-1}$. All STX analyses were made by CASAN.